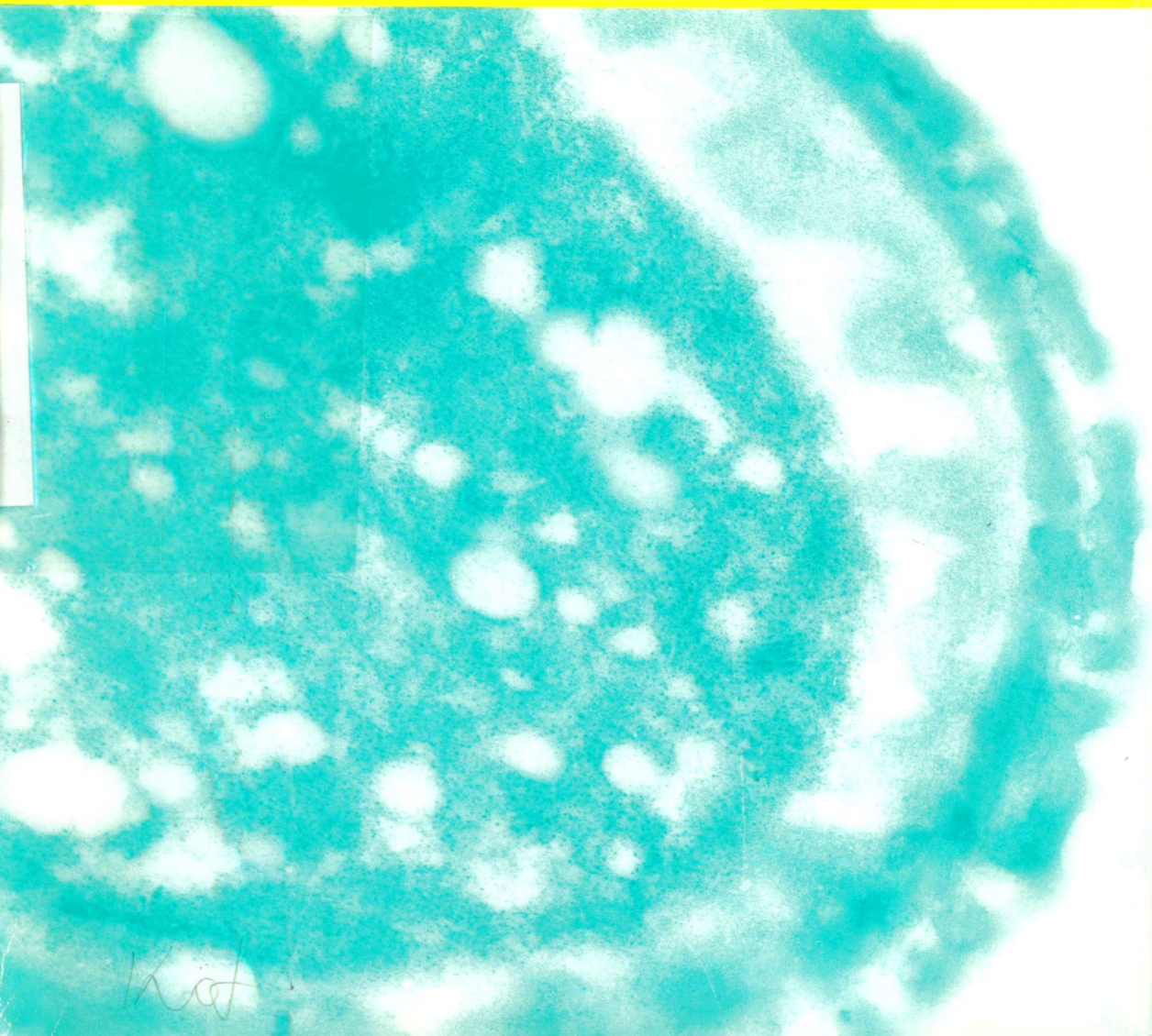
PLANT CELL BIOLOGY AND DEVELOPMENT 15



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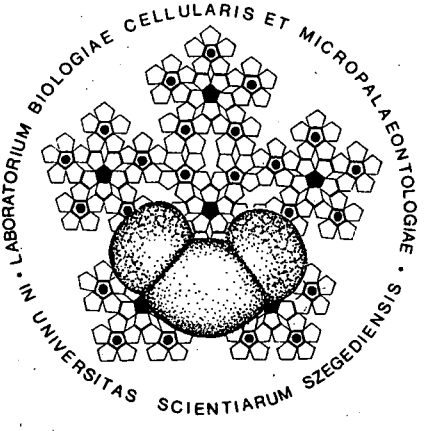
Financial support

by the Grant OTKA T 31715
by the University of Szeged
by the Faculty of Science of the University of Szeged



This volume is scientific exchange matter and not commercial

HU ISSN 0866-5443

2003
Szeged

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Preface

During last year, I received a message from Dr. Jerome EYER of the University of South Carolina, that Dr. D. ENGEHARDT passed away on February 13, 2000. His death caused a great loss in Palynology, as he has contributed excellent works on Eocene palynomorphs of U.S.A. Later, within a year Prof. Dr. h.c. Knut FAEGRI, K.St.O.O., paassed away on 10th December, 2001, at the age of 92 years. He served 56 years in the Department of Botany of the University of Bergen (Norway). He was one of the pioneer of the modern Palynology and popularized by his book "Text-book of Modern Pollen Analysis" by FAEGRI and IVERSEN (1950). This is one of the fundamental contribution in Science of Palynology, received a wide recognition and a guide reference book for the beginners.

The traditional exclusive reception of the Laboratory was held on the 19th August, 2002, but not on the date as usual. At this occasion two Commemorative Medals of the Laboratory were presented to the following personalities:

Andrea BORBOLA, a PhD. student of the Institute of Genetics of the Biological Research Center of the Hungarian Academy of Sciences. She started her works in the Laboratory as a middle-school student and continued until receiving her Diploma. Her contributions are very important in the realization of the scientific programs of the Laboratory. She has received several grants to procced her research works. A short review of her Diploma Dissertation is included herein.

Prof. Dr. Jorge Adalberto Lagos (Universidad Salvadoreña "Adalberto Mansferrer", San Salvador, El Salvador) for his important contribution in the realization of the joint research program.

Prof. Dr. A.K. SINHA Director of the Birbal Sahni Institute of Palaeobotany, Lucknow, India was awarded with the Millenium Medal of the Laboratory.

For the generous financial support of the publication of this number, I would like to express my sincere thanks:

to the Grant OTKA T 31715,

to Prof. Dr. R. MÉSZÁROS, memeber of the Hungarian Academy of Sciences, Rector of the University,

and to Prof. Dr. G. MEZŐSI, Dean of the Faculty of Science of the University of Szeged.

In consequence of new financial restrictions of the University, the Department of Botany do not assure the salary of the technician of the Laboratory. So the Laboratory is facing much difficulties but research works are still alive. In this respect a fruitful discussion was held with the Rector of the University and I hope that the Laboratory, its research and other academic activities will continue successfully with encouragements and full supports of the Rector of the University, Dean of the Faculty of Science and Grants of OTKA.

Szeged, December 2002.

M. KEDVES
Head of the Laboratory

1. EXPERIMENTAL INVESTIGATIONS ON THE TELIOSPORES OF USTILAGO MAYDIS DC

A REVIEW

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Abstract

This paper summarizes and gives a comprehensive review of the experimental results on the teliospores of *Ustilago maydis* carried out in our Laboratory by LM and TEM methods. Besides the importance of melanins, this review is about the effects of high temperature, partial degradation and X-ray irradiation of the teliospores.

Key words: Palynology, recent, *Ustilago maydis*, LM, TEM.

Introduction

The most important disease of maize is caused by smut fungi, followed by viruses, and bacteria. Of all smut genera the genus *Ustilago* most species (over 350), of which parasitize a great number of host families. *Ustilago maydis* occurs on Gramineae, especially on *Euchlaena* and *Zea*, worldwide: "Sori in stems, leaves or inflorescence forming pustules or irregular galls of considerable size, at first covered by a thin, grayish-silvery, later brown, smooth membrane which ruptures irregularly to expose the medium to dark brown, powdery spore mass. Spores globose, subglobose, ovoid to sometimes elongated or slightly irregular, 7-11 x 7-13 μm, light olive-brown; wall c. 0.5 μm thick, finely rather densely echinulate. Germination by four-celled promycelium laterally and terminally bearing basidiospores" (VÁNKY, 1985).

Ustilago maydis is well known for the extremely resistant spores, which are easily available, and occur in large masses in the infected plant. The spores retain their ability to germinate for many years. Reports of children who had fallen ill from eating bakery products, made from maize flour infected with spores of *Ustilago maydis* in 1920, in Paris (UBRIZSI, 1965). Interestingly, BUSTOS et al. (2000) and RENDUELES et al. (2000) have shown that the occurrence of the spores of *Ustilago* is moderately frequent in the air. Formers have pointed out that if the relative humidity increases, the frequency changes, for example *Phoma* and *Ustilago* become abundant.

The complex study of the biopolymer systems of the wall demonstrated that the teliospores of *Ustilago maydis* contain melanins. Therefore it is considered worth while exploring the significance of the presence of melanins.

The aim of this contribution is to give a comprehensive review of the results of experiments, which were carried out in the Cell Biological and Evolutionary Micropaleontological Laboratory.

Materials and Methods

The investigated material was collected by Dr. A. PALÁGYI on 22.8.1991. Locality: Ságvári Experimental Research Station of the Cereal Research Institute. The spores were frozen at -20°C after collection.

The experimental investigations were divided into two parts: LM and TEM methods, and included the examination of three effects: 1. High temperature, 2. Partial degradation, 3. X-ray irradiation.

1. *High temperature effect*

The high temperature effect on the spores was investigated in detail by LM method. The temperature: 200°C; length of time from 10 minutes until 300 hours: (10', 1 hr, 5 hrs, 10 hrs, 25 hrs, 50 hrs, 100 hrs, 200 hrs, 300 hrs). The slides for light-microscopical investigations were mounted in glycerine-jelly hydrated at 39.6%. 200 specimens of each sample were investigated.

2. Partial degradation

The temperature of the partial degradation was 30°C. Different organic solvents were used in LM and TEM methods. Diethylamine or merkaptoethanol were used as organic solvents. By LM 20 mg spores + 5 ml dist. water + 2 ml organic solvents, length of time: 30, 60 and 90 days. The slides for light-microscopical investigations were mounted in glycerine-jelly hydrated at 39.6%.

There were two different experiment-series with TEM. In the first series, the used organic solvent was 2-aminoethanol. There were four samples with different solvents: in the first (20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hrs), in the second (20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hrs, after washing + 10 ml $KMnO_4$ 1%, during 24 hrs), in the third (20 mg teliospores + 1 ml 2-aminoethanol, length of time 24 hrs, after washing + 10 ml $KMnO_4$ 1% during 48 hrs), and in the last one (20 mg teliospores + 1 ml 2-aminoethanol, length of time: 24 hrs, after washing + 10 ml $KMnO_4$ 1%, during 24 hrs, washing again + 2 ml acetic anhydride, length of time: 24 hrs) were used. But at the second series the used organic solvent was diethylamine. There was only one experiment (20 mg teliospores + 5 ml dist. water + 2 ml diethylamine, during 30 days). Partially dissolved spores were prepared for the TEM investigations as follows: Fixation in Millonig buffered OsO_4 1% (aq. dil.) for 1 hour. Washing in Millonig phosphate buffer overnight. Dehydration was performed in an ascending series of ethanol in 15 min. steps including uranyl acetate staining in 70% ethanol. The samples were embedded in Araldite, Durcupan (Fluka) epoxy resin in gelatine capsules and polymerized in 56°C thermostat for 3 days. The ultrathin sections were made on a Porter Blum ultramicrotome in the Electron Microscopical Laboratory of the Institute of Biophysics, at the Biological Research Center, Hungarian Academy of Sciences.

3. X-ray irradiation

The teliospores were irradiated with CuKα X-ray with BRON UM1 instrument at 35 KV, 20 mA. The length of time of the irradiation was, 5, 15, 35, 60 and 300 minutes. The irradiated spores were prepared for LM and TEM investigations. The methods of preparation for LM and TEM investigations were as described above.

The importance of melanins

Melanins are complex black polymers of resonance stabilized cyclic subunits with widely differing chemical composition, and as yet unknown molecular structure. They occur in most groups of living organisms, in the animal kingdom, plants and some fungi. "Melanins are insoluble in boiling water, hot concentrated mineral acids, and organic solvents, although they can be bleached by strong oxidizers like hydrogen peroxide, and some are degraded by treatment with strong alkalis" (BUTLER et al., 2001). In addition to the absorption of visible light, melanin absorbs gamma rays, X-rays, ultraviolet light, and infrared wavelengths, transferring energy deep into its molecular structure. It is remarkable that few researchers insist on limiting the term melanin to describe the alkali-insoluble black pigments synthesised by mammals, often called dopa melanins.

The usual sites of melanin deposition are the external parts of organisms (skin, integument and outer wall of cells), though melanin can occur in the internal organs as well. There are roles of melanins in the adaptive colouration of insects and lower vertebrates, bearing on the formation of humus in soils, in the preservation of geological record and can give data about the volume of cosmic radiation. Moreover, melanins take part in forming resistance to microbial degradation and provide a barrier against water loss under conditions of osmotic stress and also have a function as a photoprotector. Melanogenesis in animals is initiated in response to UV radiation. The protection function is not the exclusive domain of the melanins for there are other pigments which perform the same function, but apparently less efficiently, in animals, plants and fungi. "The high electron acceptor capacity of melanins, the presence of free radicals in them, and their semi-conductor properties may be related to the mechanisms of migration of energy in biological systems" (BLINOV, 1973, cited by PIROZYNSKI, 1975).

Many plant and animal disease are caused by melanized fungi. According to KUO and ALEXANDER (in PIROZYNSKI, 1975) melanized components of fungi in soils are more resistant to microbial degradation than their unmelanized components. There are lots of publications about the spreading of melanized microbes, for example higher numbers are found on rock and leaf surfaces and other exposed locations. The barrier offered by melanin may also provide protection against fungicides and heavy metals. BUTLER et al. (2001) emphasized the work of ZHDANOVA et al. (1994), who indicated that greater numbers of melanized and radiation-tolerant fungi were appearing in contaminated soils around the Chernobyl reactor in the Ukraine. But the heavy melanization is not good either, because heavy deposition of pigment may produce a very brittle wall and less resistance. RAST et al. (1981) established that the plate-like particles were of medium electron density and appeared to contain melanin in the form of granules (isolated from the spore wall of *Aspergillus bisporus*).

Results

1. LM RESULTS

At first the effect of high temperature on the spores of *Ustilago maydis* was studied by KEDVES and TÓTH (1993) with the LM method. It was observed that the outer part of the spore wall became detached at 200°C. This started after 10 hours and advanced after 100 hours of heating. The writers observed that the outermost wall layers lost spores,

and when mounted in glycerine-jelly, formed interesting patterns which they considered to be very useful in the modelling of biopolymer stuctures of the partially degraded plant cell wall.

KEDVES and GÁSPÁR (1994) observed by LM that the teliospores of *Ustilago maydis* have an extremely resistant wall after dissolving the spores with diethylamine or merkaptoethanol. They supposed that melanin took part forming considerable resistance. KEDVES and GÁSPÁR (1995) did not observe any recognizable alterations during studying the results of light microscopy of X-ray irradiated teliospores of *Ustilago maydis*.

2. TEM RESULTS

KEDVES, PÁRDUTZ and BORBOLA (1998) carried out the TEM of X-ray irradiated teliospores. The relatively low X-ray dose (5 minutes) resulted in considerable alterations to the outer part of the spore wall. There were electron-dense particles in the outer wall, moreover, an electron-dense layer could be seen at the border of the exospore and epispore. The degradation of the organelles of the cytoplasm was advanced after 15 minutes irradiation. The ultrastructure of the epispore was finely lamellar or granular. In particular at the non electron-dense granuli, the sculptural elements of exospore were destroyed. At the strongest irradiation (300 minutes) the protoplasm organelles were disintegrated and epispore and endospore were homogenized and swollen. The experiment revealed molecular units such as chains, cyclic units with pentagonal or hexagonal symmetry within the wall.

KEDVES, PÁRDUTZ and MADARÁSZ (2001) investigated the ultrastructure of partially dissoluted spores with diethylamine over 30 days. This experiment revealed the biopolymer and molecular structure of the outer wall of the teliospores. Details of the linear and for the most part cyclic molecules and highly organized globular units, similar to the Penrose-like biopolymer structures, were published. These results are similar to those of the X-ray irradiation over 300 minutes.

KEDVES and BORBOLA (2002) studied the ultrastructure of the teliospores of *Ustilago maydis* with organic solvents and oxidizing agents. Important alterations were observed at the fine structure of the wall, and in the inner part of the teliospore. The most important were as follows: the endospore swelled against 2-aminoethanol; the degradation process by 2-aminoethanol, $KMnO_4$ and acetic anhydride revealed globular biopolymer units on the outer and inner surface of the exospore. Sometimes the inner part of the endospore seemed to be more electron-dense and three layered.

Discussion and Conclusions

Differences in the composition of the biopolymer systems of the walls of different kinds of spores and pollen grains can be observed by different methods. LM results supported extreme resistance of the teliospores of *Ustilago maydis*. By TEM method, the partial degradation experiments revealed different kinds of biopolymer structures in the wall layers of the teliospores. Globular biopolymer units were observed on the surfaces. RAST et al. (1981) gave an account of similar units. The differences within samples may be the consequence of the different state of maturity of the spores.

It is rather interesting that different TEM experiments were similar or nearly the same results. For instance one of the TEM results (teliospores + 1 ml 2-aminoethanol, 24 hrs. after 10 ml $KMnO_4$ 1%, 24 hrs, and 2 ml acetic anhydride, 24 hrs) were similar

to or more or less identical with the X-ray irradiated teliospores over 15 minutes. In this way, the application of the acetic anhydride as degradation agents may be useful again for the further partial degradation experiments.

The molecular alteration of the biopolymer system is carried out by aromatization process (POTONIÉ and REHNELT, 1971). Thus the study of this kind of biopolymer structure is complicate. The presence of melanins in the wall increases complexity of the biopolymer system of the wall. Results support the assumption of the protective function of melanins against radiation, but the sporopollenin without melanins may be resistant to X-ray radiation.

Acknowledgements

The writer is thankful to Eric CAULTON director (Scottish Centre for Pollen Studies, Edinburgh, Scotland, U.K) for his valuable comments and for the linguistic corrections. I sincerely acknowledge the cooperation and letters of recommendation received from Prof. Dr. Árpád PÁRDUTZ (Institute of Biophysics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary). And last but not least I am much obliged to Prof. Dr. Miklós KEDVES (Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, Szeged, Hungary) for his active help and but for this encouragement, this review would never have been written. It was he who set me up in scientific life. Thank you once more for the patience with which you have answered my questions and for the time and trouble you have expended.

This work was supported by Grant OTKA T 031715.

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2. INVESTIGACIONES EXPERIMENTALES DE LAS ESPORAS DE ALSOPHILA SALVINII HOOKER

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Resumen

Se investigó la morfología de las esporas, frescas y experimentalmente alteradas, de *Alsophila salvinii* con el microscopico óptico (LM). En nuestro material se observó la forma básica trileta y, algunas veces, la monoleta. El protoplasma está lleno de diferentes organelas granulares. Algunos de éstos son similares a los plastidios y presentan pirenoides que son característicos de los cloroplastos de las algas. Se investigaron alteraciones como consecuencia de la hidratación, alta temperatura y los diferentes reactivos químicos. Se investigó estadísticamente la tendencia de la alteración del diámetro y la circunferencia de las esporas. Las esporas de esta especie son, en general, relativamente resistentes a las influencias experimentales.

Introduccion

Existe mucha información sobre la presencia de las esporas de los helechos arborescentes en épocas geológicas pasadas, pero este interesante helecho es un constituyente importante de la presente vegetación tropical.

Hay muchas opiniones en torno a la taxonomía de este helecho, por ejemplo HARRIS (1955) en su libro enfatize lo siguiente, p. 97: "El unico género que aquí se discute es *Cyathea*, en al cual están incluidas las especies que antes eran referidas a *Alsophila* y *Hemitelia*". Posteriormente WELMAN (1970) menciona el género *Cyathea* SM. que comprende alrededor de 800 especies encontradas en las regiones tropicales. Hay muchas publicaciones relacionadas con la morfología de esporas recientes y fósiles de este género de helecho. Entre las más importantes monográfías citamos las de TRYON y LUGARDON (1991). Ellos estudiaron 34 especies del género *Alsophila* R. BROWN. Se publicaron las microfotográfías electrónicas de barrido (SEM) de *A. minor* (D.C. EATON) TRYON, *A. capensis* (L.F.) J.S.M., *A. dregei* (KZE) TRYON, *A. bryophyla* x *Nephelea portoricensis, A. caudata* HOOK., *A. podophylla* HOOK., *A. tricolor* (COLENSO) TRYON, *A. decurrens* HOOK., *A. bryophyla* TRYON. De *A. bryophila* fueron publicadas la ultraestructure de la pared.

Durante las investigaciones de aeropalinología en Taipei, CHEN y HUANG (1980) mencionaron la presencia de esporas de helechos en la atmósfera. HUANG (1998) incluide en su monográfía, como esporas anemófilas,del helecho arborescente *Cyathea lepifera.*

LÖTSCHERT (1959) y SEILAR (1980) señalan la presencia de *Alsophila salvinii* en El Salvador. WETTSTEIN (1944) y ENGLER (1954) confirman la existencia del género *Alsophila* y mencionan otras especies. *Alsophila salvinii* HOOKER es un helecho arbores-

cente que alcanza hasta 7 metros de altura; se distribuye en altitudes entre 700 y 2500 metros: las hojas son grandes, alcanzando hasta 1.50 m. de longitud, bipinnadas y son soros sin induiso en el envés de las pínnulas. Crece, con frescuencia, en los bosques nebulosos.

Tomando en consideración la importancia de las esporas, hemos iniciado un programa de investigaciones sobre estudios experimentales de *Alsophila salvinii*. En. el presente trabajo se has resumido los primeros resultados con el microscopico óptico (LM).

Materiales y Metodos

El material para la investigación fue recolectado por el primer autor
Los experimentos son como sigue:
T-9-P-73 esporas frescas montadas en gel de glicerina: T-9-P-81 tratadas con solución KJ.J.
3 mg de esporas + 5ml de agua destilada a 30°C durante 24 horas (T-9-P-78) y 120 horas (T-9-P-79).
5 mg de esporas + 1 ml de 2-aminoetanol a 30°C durante 24 horas (T-1/7-1300), 48 horas (T-1/7-1321) y 72 horas (T-1/7-1322). T-1/7-1323 comenzando como T-1/7-1300, después de lavarlas + 10 de $KMnO_4$ al 1%. T-1/7-1324 comenzando como T-1/7-1321, T-, T-1/7-1325 comenzando como 1322 después lavar + 10 ml $KMnO_4$
3 mg de esporas calentadas a 200°C durante 10 minutos (T-9-P--74), 1 hora (T-9--P-75), 5 horas (T-9-P-76), 10 horas (T-9-P-77) y 25 horas (T-9-P-80). Las esporas fueron montadas en gel de glicerina hidratada al 39.6% (LOBREAU, 1966), después de los experimentos.

Se investigaron las siguientes características:

1 - El tamaño y el diámetro de las esporas.

2 - La distribución de las esporas en posición polar y ecuatorial.

3 - La esporádica presencia de la forma monoleta entre la característica forma trileta dominante.

4 - El aspecto de las caras triangular, convexa y cóncáva.

5 - Finalmente la forma triplanoide y triplana.

Debemos de enfatizar, después de PFLUG (1953), que las alteraciones trilete - triplana - poroplana son importantes en la evolución de los granos de polen de las primitivas angiospermas de Europa, para la taxa del grupo de los Normapolles. Diferentes investigaciones se han publicado sobre las variaciones morfológicas de las esporas triletas, por ejemplo: SLADKOV (1957, 1959a,b, 1961, 1962), DEÁK (1959), KEDVES (1960, 1961). M. VAN CAMPO (1961) investigado las alteraciones secundarias del área de las aperturas. El objetivo de su investigación fue lo relacionado con el aparecimiento del toro (kyrtom) alrededor de las bifurcaciones de los ángulos de la tétrada en las esporas triletas. HUANG (1981) ha recopilado, en un resumen muy útil, lo relaciondao con la morfología básica obtenida con el microscopico óptico (LM) en la que incluye la variedad de las formas de las espóras de las Pteridófitas.

Resultados

En los cuadros siguientes se exponen las diferentes clases de alteraciones a consecuencia de los experimentos.

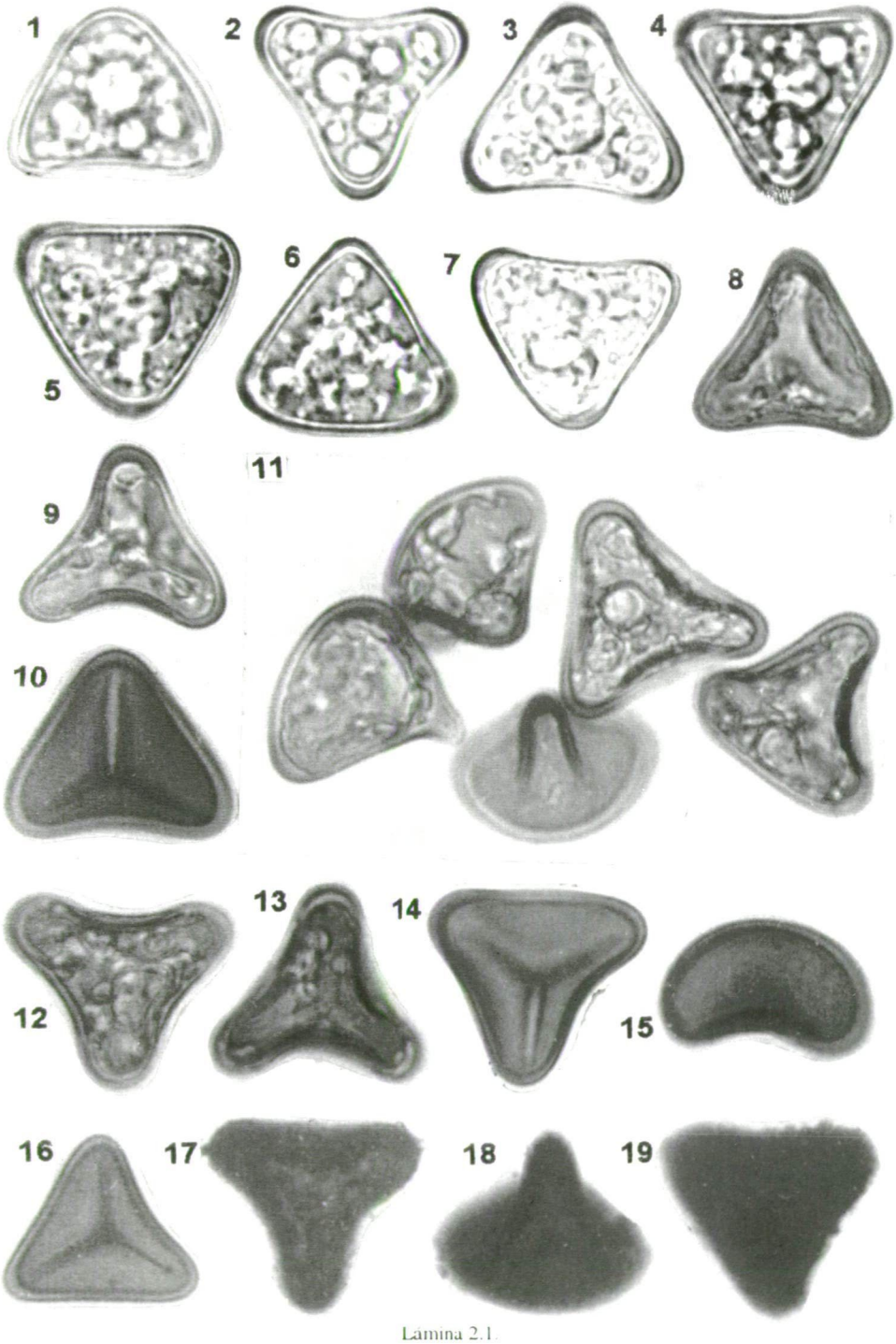
A - Diámetro de las esporas en posición polar.

Numero del experimento	Tamaño (µm %)								Tamaño dominante (µm)	Promedio (µm, %)
	22.5	25	27.5	30	32.5	35	37.5	40		
T9-P-73			2.0	9.5	25.5	38.0	22.5	2.5	35.0	34.43
T9-P-81			0.5	9.0	22.0	40.5	27.0	1.0	35.0	34.68
T9-P-78			1	11.5	27.5	42	17.5	0.5	35	34.13
T9-P-79			4	20	**29**	**33**	14		32.5; 35	33.32
1/7-1320	0.5	2.0	13.5	20.0	39.5	22.0	2.5		32.5	31.8
1/7-1321		5.5	23.5	29.5	24.0	16.0	1.5		30.0	30.68
1/7-1322		7.0	25.5	21.0	22.5	19.5	4.5		27.5	30.88
1/7-1323		1.5	7.5	21.5	30.0	22.5	16.5	0.5	32.5	32.9
1/7-1324			3.5	16.0	23.0	29.5	26.0	2.0	35.0;37.5	34.1
1/7-1325			3.5	12.0	24.0	28.5	30.0	2.0	35.0;37.5	34.38
T9-P-74			3.0	20.0	30.5	28.0	18.0	0.5	32.5;35.0	33.48
T9-P-75		1.0	14.5	30.5	26.5	20.5	7.0		30.0;32.5	31.8
T9-P-76		2.5	26.0	26.5	25.5	14.5	4.5	0.5	27.5;30.0;32.5	30.98
T9-P-77		2.5	17.5	32.0	26.5	16.0	5.5		30.0;32.5	31.3
T9-P-80			8.5	32.0	32.5	23.0	4.0		30.0;32.5	32.05

1 - El tamaño de las esporas frescas (T-9-P-74, T-9-P-81) y las esporas hidratadas durante 24 horas son más o menos iguales. La tendecia a la disminución del diámetro se presenta después de 120 horas de hidratación..
2. - El máximo tamaño disminuyó con el tratamiento con 2-aminoetanol durante 24, 48, y 72 horas (Experimentos No.: T-9-P-74, 75, 76). Se observó una notable tendencia a expandirse cuando las esporas fueron tratadas con 2-aminoetanol y $KMnO_4$.
3 - Los resultados con la alta temperatura fueron más o menos regulares, pero no característicos, en la disminución del diámétro de las esporas.

B - Variedad de formas.

	polar					equatorial	monoleta
	triangular	convexa	cóncava	triplanoide	triplane		
T9-P-73	15.5	12.5	18.0	2.0		53.0	
T9-P-81	17.0	2.0	14.0	20.5		46.5	
T9-P-78	16	9	21	8	1.5	44.5	
T9-P-79	8	2.5	18	7.5		3.5	0.5
1/7-1320	6.0	5.5	19.5	49.0	4.0	16.0	
1/7-1321	7.5	4.0	25.5	37.0	2.0	24.0	
1/7-1322	4.0	2.5	29.0	38.0	0.5	25.0	1.0
1/7-1323	6.5	2.5	33.0	38.5	1.0	17.5	1.0
1/7-1324	10.5	3.5	31.0	30.5		24.0	0.5
1/7-1325	10.0	6.0	34.5	31.0	2.0	16.5	
T9-P-74	6.0	2.0	26.0	16.0		50.0	
T9-P-75	2.5	2.0	31.5	22.5		41.0	0.5
T9-P-76	0.5	2.5	28.0	41.0	0.5	27.0	
T9-P-77	1.5	0.5	25.0	54.0		19.0	
T9-P-80	6.0	0.5	20.0	40.0	3.5	29.5	0.5



Lamina 2.1

Lámina 2.1.

1-19. *Alsophila salvinii* HOOKER
1-3. Esporas frescas.
4,5. Esporas hidratadas durante 1 dia.
6,7. Esporas hidratadas durante 5 dias.
8-11. Esporas disueltas parcialmente en 2-aminoetanol durante 24 horas.
12-15. Esporas disueltas parcialmente en 2-aminoetanol durante 48 horas.
16. Esporas después de disolución parcial en 2-aminoetanol durante 72 horas.
17. Esporas parcialmente degradadas con 2-aminoetanol durante 24 horas y exidadas con dilusión acuosa de $KMnO_4$ al 1 % durante 24 horas.
18. Esporas parcialmente degradadas con 2-aminoetanol durante 48 horas, y oxidadas con dilusión acuosa de $KMnO_4$ al 1 % durante 24 horas.
19. Esporas parcialmente degradadas con 2-aminoetanol durante 72 horas y oxidadas con dilusión acuosa de $KMnO_4$ al 1 % durante 24 horas. 1.000x.

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1 - Las formas monoletas fueron observadas en pequeña cantidad durante estas investigaciones.

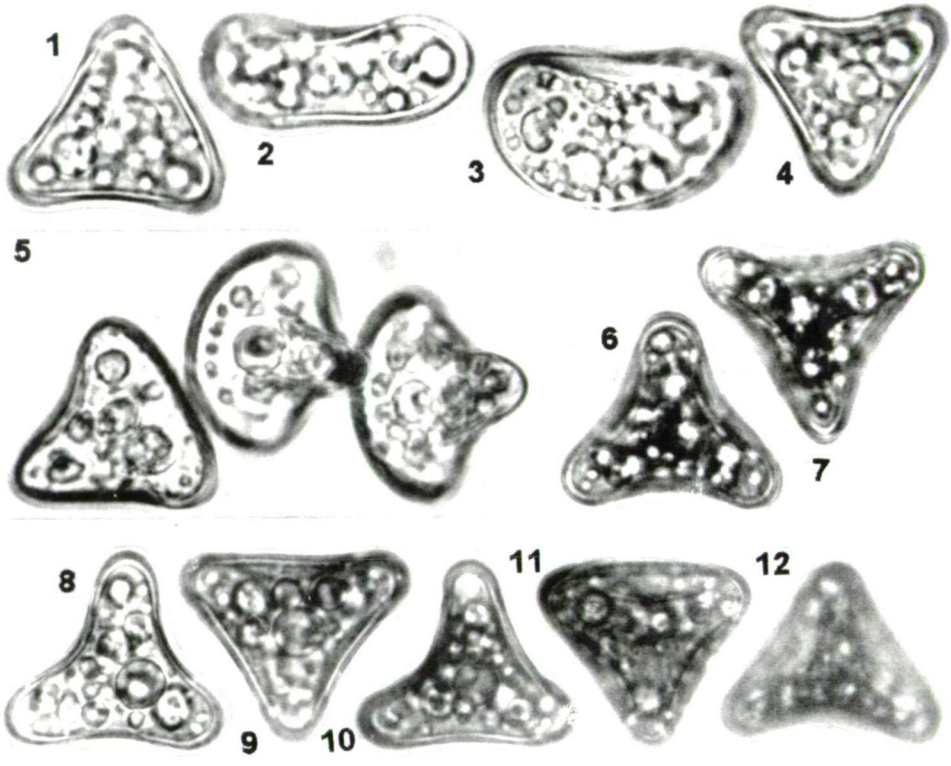

Lámina 2.2.

1-12. *Alsophila salvinii* HOOKER
1-3. Esporas calentadas a 200°C duriante 10 minutos.
4,5. Esporas calentadas a 200°C durinte 1 hora.
6-8. Esporas calentadas a 200°C durante 5 horas.
9,10. Esporas calentadas a 200°C durante 10 horas.
11,12. Esporas calentadas a 200°C durante 24 horas. 1.000x.

2 - Las formas triplanas no fueron observadas en esporas frescas. Durante los experimentos la cantidad es como máximo, menor al 4.0% y no ocurre siempre en los experimentos usados.
3 - La gran cantidad de formas triplanoides fue el resultado de la degradación parcial con 2-aminoetanol; después del tratatamiento con 2-aminoetanol y $KMnO_4$ se incrementaron las formas triangulares y cóncavas.
4 - Después de los efectos de las altas temperaturas son dominantes, en general, la forma cóncava y, algunas veces, la forma triplanoide.

Discusion y Conclusiones

De acuerdo con nuestros nuevos resultados obtenidos en las esporas de *Alsophila salvinii* HOOKER, podemos afirmar lo siguiente:

1 - Con toda probabilidad hay pirenoides en los plasticidios de estas esporas. Para obtener una mayor y definitiva información necesitamos realizar investigaciones con el microscopio electrónico de transmisión.
2 - La poca y esporádica presencia de formas monoletas, comprueba el tipo primitivo de espora.
3 - La morfología de las esporas, investigadas con el microscopio óptico, es relativamente resistente a la acción de las diferentes classes de experimentos. Lo mismo puede establecerse en los experimentos de degradación con 2-aminoetanol y $KMnO_4$.
4 - Se están realizando nuevos estudios experimentales que incluyen la solución de C60 Fullereno/benzol, los cuales serán objeto de nuevas publicaciones.

Agradecimiento

Estos trabajos fueron patrocinados por la Grant OTKA T 031715.

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Erratum, P.C.B.D. 14, p. 59/60, recte: Durante las operaciones de simetria, el pentágono regular de la unidad biopolímera de la ectexina, parcialmente degrada del polen del ragweed, se rotó un pentágono extremadamente degradado y, por primera vez, fue publicada la estructura molecular de la unidad biopolímera globular (KEDVES, PÁRDUTZ y MADARÁSZ, 2002), lo cual fue un paso importante para los estudios del esqueleto metaesteble cuasi-cristaloide de la ectexina.

3. MEGASPORES FROM SANDY SHALES ASSOCIATED WITH A LOCAL COAL SEAM EXPOSED IN THE VICINITY OF HAHAJOR VILLAGE, HURA TRACT, RAJMAHAL BASIN, INDIA

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Abstract

Megaspores are recorded from sandy shales associated with the local coal seam exposed in shallow pits in the vicinity of Hahajor Village, Godda District, Jharkhand. Almost all the megaspores are of apiculate type and represent the genera *Biharisporites* (2 spp.), *Jhariatriletes* and *Singhisporites* (1 sp. each)

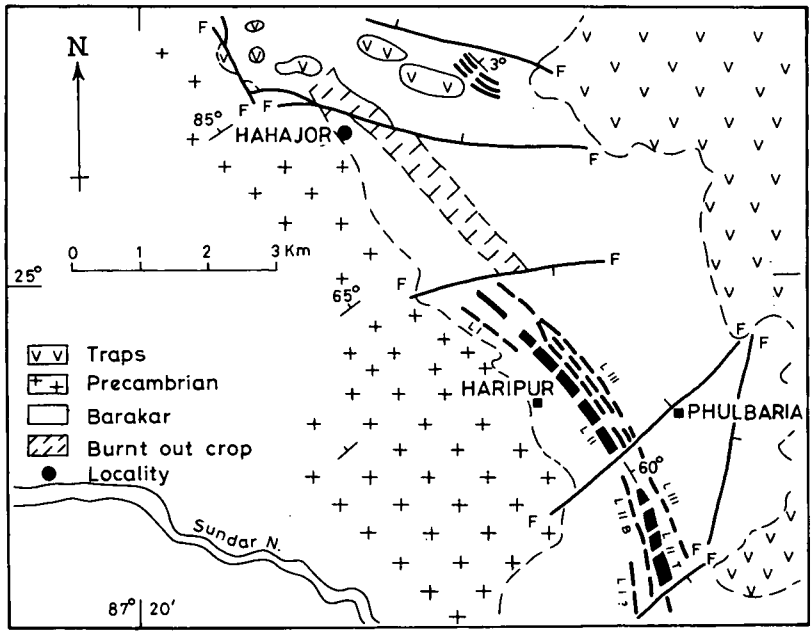
Key words: Palynology, megaspores, Barakar Fm., Rajmahal Basin, India, SEM

Introduction

A major part of the Rajmahal Basin is covered with the Rajmahal Traps with intermittent exposures of the fossiliferous inter-traps of Early Cretaceous Age. A narrow strip, on the western side of the basin and running the full length north to south, shows exposures of Gondwana sediments, mostly of Barakar Age. The northernmost outlier of these sediments contains the Lalmatia Coal Seam, which is the major coal-productive horizon in the basin (RAJA RAO, 1987). Besides the official Lalmatia Open Cast Mining Project, coal is periodically taken out by local villagers from shallow pits dug by them, particularly in the vicinity of Hahajor, Haripur and Hura Villages (Text-fig. 3.1.). These coal occurrences may also represent one of the three Lalmatia Coal Seams.

FEISTMANTEL (1881) first reported plant fossils from the area. These included a few leaves of the genus *Glossopteris*, some seeds and scale leaves. Almost a hundred years later, SINGH, SRIVASTAVA and MAHESHWARI (1986) recorded a heterophyllous sphenophyll *Sphenophyllum gondwanensis* and the equisetale *Lelstotheca* from the grey shales associated with the Lalmatia Coal Seam. Later, a much diversified flora, comprising species of the genera *Phyllotheca, Lelstotheca, Sphenophyllum, Trizygia, Vertebraria, Glossopteris, Ginkgoites, Psygmophyllum, Rhipidopsis, Saportaea, Birbalsahnia, Veekaysinghia* and some fern taxa, was recorded from the fawn, grey and black shales associated with the Lalmatia Coal Seam in different parts of the basin (BAJPAI and MAHESHWARI, 1991, BAJPAI, 1992, MAHESHWARI and BAJPAI, 1992; unpublished field data). MAHESHWARI and BAJPAI (1990) reported the presence of trace fossils in association with the plant megafossils from the Haripur area. Pollen and spores have also been reported particularly from the sub-surface sediments.

During one of the field trips to the area, we collected a few impressions of equisetalean and *Glossopteris* leaves with the weathered carbonified crust in the sandy shales associated with a local coal seam, which was being unofficically exploited by the local villagers near Hahajor Village (Text-fig. 3.1.). Chemical processing of the carbonified crust did not yield any cuticle. However, the maceration of the shales yielded a rich, though not much diversified, haul of megaspores. Identifiable taxa of these megaspores are reported in this paper.



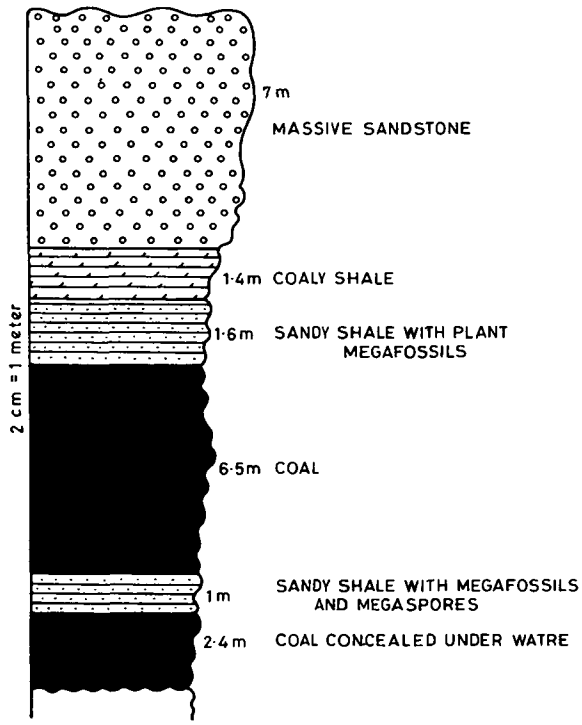
Text-fig. 3.1.

Map of the investigated area.

Materials and Methods

In the shallow pit dug near Hahajor Village, the coal was found at a depth of about 19.5 meters (Text-fig. 3.2.). The overburden comprised mostly sandy and sandy/ ferruginous shales. The sandy shale containing plant megafossils was sampled. Some samples were also collected for bulk maceration. The latter were treated with hydrofluoric acid for three to four weeks to digest the silica. The washed residue contained a very large number of megaspores, but only a few pollen and spores. The megaspores were individually picked up with a fine sable-hair brush. The megaspores were sorted under the low power binocular microscope. The megaspore population was divided into two parts, one for controlled maceration for the study of the inner structure, and the other for scanning electron micrography for study of the surface ornamentation.

For the study of the inner body (mesosporium) the megaspores were macerated in concentrated nitric acid and digested in very diluted alkali (KOH) at different stages of maceration (HØEG, BOSE and MANUM, 1955, PANT and SRIVASTAVA, 1961, BHARADWAJ and TIWARI, 1970, TEWARI and MAHESHWARI, 1992). Photomicrographs were taken at different stages of transparency. For scanning electron micrography, megaspores were dehydrated in ethanol series and then dried. The dried megaspores were mounted on aluminium stubs with silver tape, coated with gold and studied under LEO 430 Scan.



Text-fig 3.2.

Lithology of the sample site near Hahajor village

Results

Genus: *Biharisporites* POTONIÉ emend. BHARADWAJ and TIWARI 1970
Biharisporites boralii sp. nov (Plate 3.1., figs. 1,2)

Diagnosis and description. – Megaspores trilete, circular in proximal-distal view: diameter 550-670 μm in dry condition and 720-810 μm in wet condition. Trilete laesurae raised, prominent almost straight, about two-thirds of radius long. Arcuate ridges uniformly thick, clearly outlining the contact areas. Surface ornamentation of exine spinate under light microscope, spines very distinct under scanning electron microscope, differ in size and form; some with blunt tips even look like baculae. Some of the spines, particularly those in the equatorial region having highly furcated tips. Ornamentation much reduced in contact areas. Fractured surface of the exine showing usual sporopollenin units. Mesosporium distinct and without cushions.

Comparison. – The nature of the spines, particularly of those in the equatorial region distinguishes this species from other known species of the genus. In *Biharisporites robustus* PANT and MISHRA 1986, the spines have pointed tips, while in *B. (Triletes) spinulosus* (SINGH 1953) POTONIÉ 1956, the spine tips are blunt.

Holotype. - Plate 3.1., fig. 1, SEM Stub no.: 1, Birbal Sahni Institute of Palaeobotany, Lucknow.

Derivatio nominis. - After late Mr. H.N. BORAL, Technical Officer at Birbal Sahni Institute of Palaeobotany, who was a great help both in the field and the laboratory.

Biharisporites ghoshii sp. nov. (Plate 3.1, figs. 3-5)

Diagnosis and description. – Megaspores trilete, azonate, apiculate, almost circular in outline in proximo-distal view. Megaspore diameter 495-525 µm in dry condition and 655-750 µm in wet condition. Trilete laesurae imperceptibly sinuous and raised, three-fourths of spore equator in length. Due to weak arcuate ridges contact areas not sharply defined. Both the proximal and distal surfaces covered with tiny spines, with strong bases; spines very prominent in the equatorial region, tips of the spines often blunt. Spongy mesh of sporopollenin units visible in slightly over-macerated specimens. Mesoporium without cushion.

Comparison. – The species is distinguished by its characteristic surface ornamentation.The species closely compares with *Biharisporites spinulosus* (SINGH 1953) POTONIÉ 1956, but can be distinguished by its weak arcuate ridges and indefined contact areas.

Holotype. - Plate 3.1., fig. 4, SEM stub no.: 1., Birbal Sahni Institute of Palaeobotany, Lucknow.

Derivatio nominis. - After late Professor A.K. GHOSH, one of the pioneer palynologists of the country.

Genus: *Jhariatriletes* BHARADWAJ and TIWARI 1970

Jhariatriletes bharadwajii sp. nov. (Plate 3.1., figs. 6,8, plate 3.2., figs. 2-4)

Diagnosis and description. - Megaspores trilete, azonate, apiculate. Megaspore diameter 415-545 µm in dry condition and 495-695 µm in wet condition. Trilete laesurae strong, raised and extend for about three-fourth of the spore radius. Arcuate ridges not seen leaving ill-defined contact areas. The surface ornamentation appears baculate under the light microscope, but at greater magnification, under the scanning electron microscope, the individual elements appear to be hollow, with expanded bases, and open furcate tips. At one place, a small microspore seen entrapped in the maze of these appendages. Mesosporium without cushions.

Comparison. - The characteristic hollow appendages distinguish this species from *Jhariatriletes baculosus* BHARADWAJ and TIWARI 1970 and *J. srivastavae* BHARADWAJ and TIWARI 1970.

Holotype. - Plate 3.1., fig. 9, SEM Stub. no.: 1, Birbal Sahni Institute of Palaeobotany, Lucknow.

Derivatio nominis. - After late Dr. DINESH CHANDRA BHARADWAJ, who established the school of Coal Palynology at the Birbal Sahni Institute of Palaeobotany, Lucknow.

Genus: *Singhisporites* POTONIÉ emend. BHARADWAJ and TIWARI 1970

Singhisporites pantii sp. nov. (Plate 3.1., fig, 7, plate 3.2., fig. 1)

Diagnosis and description. - Megaspores trilete, azonate, apiculate. Megaspore diameter 327-410 µm in dry condition and 425-510 µm in wet condition. Trilete laesurae thick, raised straight, almost reach the spore equator. Arcuate ridges not seen, and hence

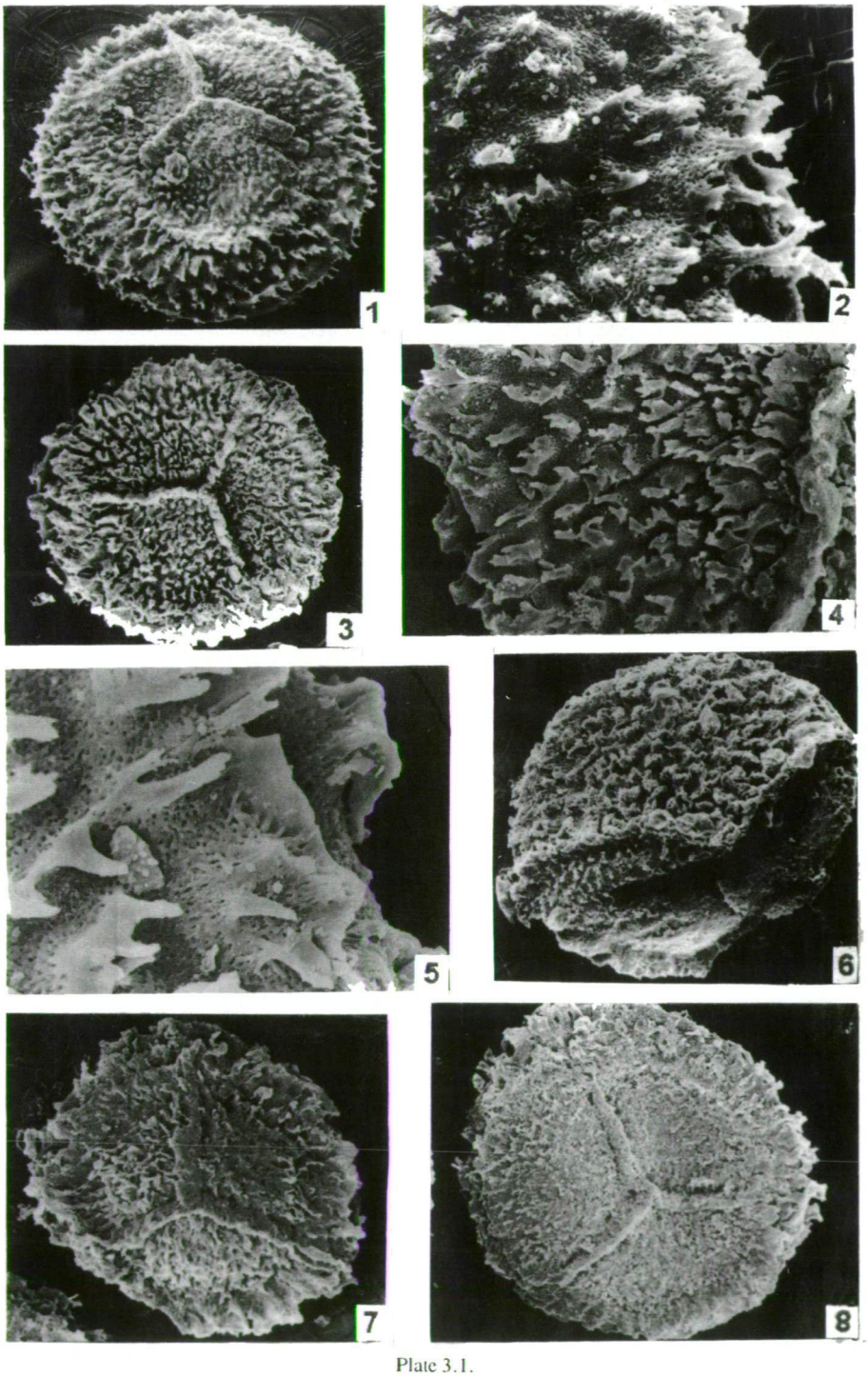


Plate 3.1.

Plate 3.1.

1. *Biharisporites boralii* sp. nov., type specimen. BSIP SEM stub no.: 1, 200x.
2. *Biharisporites boralii* sp. nov., details of exine sculpture near and at the equator, 2.500x.
3. *Biharisporites ghoshii* sp. nov., type specimen, BSIP SEM stub no.: 1, 160x.
4. *Biharisporites ghoshii* sp. nov., details of ornamentation of the exine from the trijunction of the equator, 1.500x.
5. *Biharisporites ghoshii* sp. nov., the megaspore sporoderm at a greater magnification to show the varying shapes of the spines and the spongy texture, 9.500x.
6. *Jhariatriletes bharadwajii* sp. nov., a specimen showing sporoderm ornamentation on the distal surface, 370x.
7. *Singhisporites pantii* sp. nov., type specimen, BSIP SEM stub no.: 1, 270x.
8. *Jhariatriletes bharadwajii* sp. nov., type specimen, BSIP SEM stub no. 1, 360x.

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contact areas not clearly demarcated. Exine densely ornamented with appendages of various shapes and types, ornamentation even clear on the contact faces. Ornamentation however, more developed on the distal face and on the equatorial zone. Equatorial appendages thick, comparatively close and fleshy almost giving a zonate appearance.

Comparison. - The species is distinguished by its equatorial appendages, which give the megaspore a 'zonate' appearance. Comparable species *Singhisporites radialis* BHARADWAJ and TIWARI 1970, *S. (Trilites) surangei* (SINGH 1953) POTONIÉ 1956 and *S. (Mammilaespora) waltonii* (PANT and SRIVASTAVA 1962) BHARADWAJ and TIWARI are larger in size being up to 1.000 µm in diameter.

Holotype. - Plate 3.1., fig. 10, SEM Stub no.: 1., Birbal Sahni Institute of Palaeobotany, Lucknow.

Derivatio nominis. - After late Professor DIVYA DARSHAN PANT, renowned plant biologist and palaeobotanist.

Discussion and Conclusions

Two types of megaspores, non-apiculate and apiculate, have been found in abundance in the sandy studies. The non-apiculate types do not show very distinctive characters and are referable to the *Banksisporites/Srivastavaesporites*-complex. The apiculate megaspores are referred species of the genera *Biharisporites, Jhariatriletes* and *Singhisporites.* These megaspores are believed to be those of heterosporous free-sporing lycopods (PANT and MISHRA, 1986). However no lycopod has so far been recorded from these shales or equivalent sediments in the Rajmahal Basin.

The megaspore genera *Biharisporites* and *Jhariatriletes* are long ranging: *Biharisporites* occurs in sediments of "Karharbari" ro Tiki Formations (BAJPAI, 1992, PAL, GHOSH and SANNIGRAHI, 1997). The other genus, namely *Singhisporites* is so far known only the Barakar, Kulti and Raninganj Formations (MAHESHWARI and TEWARI, 1987).

Acknowledgements

I place on record my thanks to Prof. A.K. SINHA Director, Birbal Sahni Institute of Palaeobotany, Lucknow for evincing keen interest in the progress of the work and arranging necessary facilities. I am grateful to Dr. H.K. MAHESHWARI, former Deputy Director, BSIP for his valuable advice. Mr. V.K. SINGH assisted with scanning electron micrography, and Mr PRADEEP MOHAN prepared final photoprints. I greatly appreciated their help.

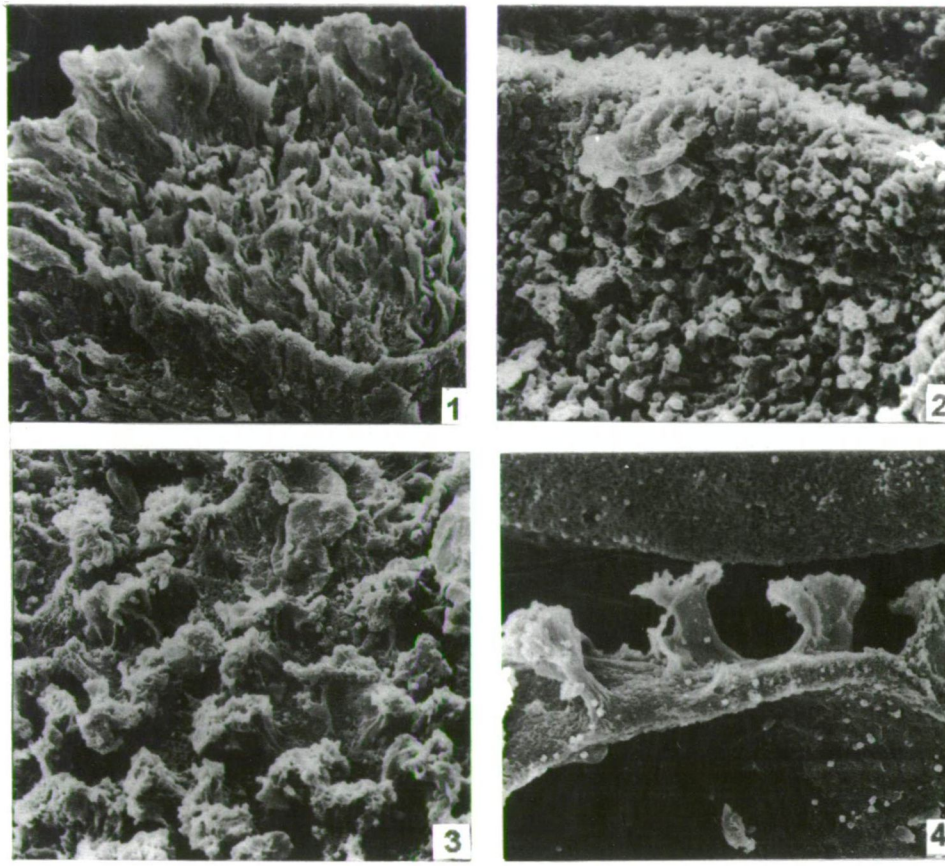

Plate 3.2.

1. *Singhisporites pantii* sp. nov., details of sporoderm sculpture in one of the proximal contact faces and at the equator, 1.800x.
2. *Jhariatriletes bharadwajii* sp. nov., details of surface ornamentation on the distal surface of the megaspore, 9.000x.
3. *Jhariatriletes bharadwajii* sp. nov., a microspore is seen entangled in the ornament elements on the proximal face, 6000x.
4. *Jhariatriletes bharadwajii* sp. nov., the characteristic hollow appendages at the equatorial region, 6.500x.

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4. LM, SEM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED POLLEN GRAINS OF CYCAS RUMPHII MIQ. FROM INDIA

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Abstract

Results of different experiments carried out on pollen grains of *Cycas rumphii* MIQ. with use of 2-aminoethanol, $KMnO_4$ aq. dil. and merkaptoethanol have been summarized. Alterations in morphology of the studied pollen were observed with the help of light microscope and the superficial degradation was studied with SEM. Ultrastructure of partially degraded exine with 2-aminoethanol and $KMnO_4$ was investigated with a view to observe the changes or alterations. Ultrastructural studies reveal that no further thinning or reduction in ectexine was noticed in apertural area of pollen. Molecular structures of different organization levels were observed in highly magnified pictures of the pollen wall.

Key words: Experimental Palynology, recent *Cycas rumphii*, LM, SEM and TEM.

Introduction

Cycadales are among the important constituents of the Mezozoic vegetation all over the world and are significant in the present day vegetation of the tropical regions. Large number of publications concerning the fossil cycadalean taxa, e.g.: *Cycadopites* (WODEHOUSE 1933) ex WILSON and WEBSTER 1946 COOKSON (1947), *Ginkgocycadophytus* SAMOILOWICH 1953, *Cycadaceaelagenella* MALYAVKINA 1953, COUPER (1953), cf. POTONIÉ (1958), KRUTZSCH (1970) have appeared. Monosulcate gymnosperm pollen are very significant from the point of view of the evolution of angiosperms particularly in the Normapolles Province (DOYLE, 1977, HICKEY and DOYLE, 1977, etc.).

Many publications have appeared concerning the pollen grains of the recent Cycadales. Reference to some of these is as follows: GULLVÅG (1966, p. 444) pointed out the following: "WODEHOUSE took as starting-point the type of pollen grain which he regards as most primitive, viz., the type found in the *Cycadales* and in *Ginkgo* WODEHOUSE (1933) mentioned "This one-furrowed or monocolpate type of grain besides occurring throughout the Cycadales, is characteristic of many monocotyledons and primitive dicotyledons, e.g. the Palmaceae, Magnoliaceae and Nymphaeaceae. Its great stability and resistence in these divergent groups are in keeping with its antiquity."

Light microscopic studies on morphology of *Cycas rumphii* pollen grains ERDTMAN (1965, p. 30) made the comments as follows: "*C. rumphii* (Borneo:, Clemens 21173) 17,5 x 20 x 26 µ. Proximal face with OL pattern probably indicating a fine reticulation." HUANG (1972) described the pollen grains of the *Cycas* genus as being 1-colpate or rarely 1-colporate. Pollen grains of Cycadaceae were noticed to be boat shaped, with single sulcus on the distal face and vermiculate or perforate exine (XI YI-ZHEN and

WANG FU-HSIUNG, 1989). The surface was observed to be psilate to finely reticulate with OL pattern (ERDTMAN, 1965. HUANG, 1972), fossulate (WANG, 1990), vavermiculate or perforate (XI YI-ZHEN and WANG FU-HSIUNG, 1989), foveolate (XI YI-ZHEN, 1990). The absence of the comfit perine in Cycadaceae was established by UENO (1960). Tetrad formation and the sugar content were investigated by UENO (1960, 1982). Dry and hydrated pollen grains of the genus *Encephalartos* and *Ceratozamia* were investigated by KEDVES, BORSODI, DOBÓ, KOVÁCS and SZÉCSÉNYI (1999a,b).

SEM studies on pollen of *Cycas revoluta* revealed that surface is foveolate and diameter of the foveolae is differential (ERDTMAN, 1965). YAMAZAKI and TAKEOKA (1962) described that this species is dotted with many irregular pit-like concavities (longest diameter about 0.2-0.8 μ) and areas between the concavities are marked with a very faint wave-like pattern. Further SEM data: TAKEOKA (1965), and UENO (1978, *Cycas taiwaniana*).

Many reports concerning the ultrastructure of the exine of the extant Cycadaceae pollen have been published (AFZELIUS, 1956, GULLVÅG 1966, AUDRAN, 1970, 1974, 1978b, 1979a,b, 1980, 1981, 1986), AUDRAN and MASURE (1976a,b,c, 1978), XI YI-ZHEN and WANG FU-HSIUNG (1989), XI YI-ZHEN (1990). Transformation in the ultrastructure of cycads pollen grains by acetolysis and $KMnO_4$ was established by AUDRAN (1978a).

TEM studies on fossil cycadaceous exines were published by TREVISAN (1980) and ZAVADA and DILCHER (1980) and KEDVES (1985, 1994).

DEHGAN and DEHGAN (1988) investigated pollen grains of several species from extant Cycadaceae with LM, SEM and TEM methods.

Biopolymer organizations on the intine was first investigated on the partially degraded pollen grains of *Encephalartos ferox* (KEDVES, 1991). The biopolymer structure and the symmetry in partially degraded exines of Cycadaceae were investigated by KEDVES, PÁRDUTZ, TERBE and HORVÁTH (1999).

Pollen grains and immature and mature leaves of *Cycas rumphii* were investigated by employing similar experiments with an object to observe the degradation in sporopollenin and cuticle.A preliminary report is presented in this number (KEDVES, PRISKIN, TRIPATHI and MADHAV KUMAR).

In this communication we have presented the results of experiments on pollen grains and have compared them with those observed in monocolpate palm pollen from India.

Materials and Methods

The investigation material was collected by Dr. MADHAV KUMAR from the garden of the Birbal Sahni Institute of Palaeobotany, Lucknow, India on the 23rd January, 2001.

Polar axis and P/E ratio were investigated statistically.

Dry and experimentally degraded pollen grains were investigated.

The fresh pollen grains on the slides were covered by a cover glass which was fixed on the corner with small drops of glycerine. In this way we have the opportunity to take the pictures with an objective of oil immersion from unstained dry pollen grains. Unstained (A) and stained with Methylviolet (B) pollen grains mounted in glycerine-jelly were investigated (T-12-244).

Pollen grains were hydrated for 24 hours at 30 °C, (T-12-245) and unstained (A) and stained (B) pollen grains were investigated.

Partial degradations: (temperature: 30 °C)

T-12-148. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 24 hours.

T-12-149. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 48 hours.

T-12-150. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 72 hours.

After washing the partially degraded pollen grains were investigated with the LM and SEM. The SEM photographs were taken in the SEM Laboratory of the Birbal Sahni Institute of Palaeobotany, Lucknow, India on LEO 430 Scan instrument, resolution 40 Å.

T-12-151. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 24 hours, washing, + 10 ml $KMnO_4$, 1%, length of time: 24 hours.

T-12-152. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 48 hours, washing + 10 ml $KMnO_4$ 1%, length of time: 24 hours.

T-12-153. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 72 hours, washing + 10 ml $KMnO_4$ 1%, length of time: 24 hours.

Partially degraded pollen grains were investigated with the LM, SEM and TEM. The ultrathin sections were made in the Cell Biological and Evolutionary Micropaleontological Laboratory on a Porter Blum ultramicrotome with glass knives. The TEM photographs were taken in the EM Laboratory of the Department of Biophysics of the Biological Reseach Center on a Tesla BS 540 instrument resolution about 6-7 Å, and a Zeiss Opton EM-902,resolution 2-3 Å.

T-12-154. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 24 hours, washing, + 2 ml merkaptoethanol, length of time: 24 hours.

T-12-155. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 48 hours, washing + 2 ml merkaptoethanol, length of time: 24 hours.

T-12-156. 10 mg pollen grains + 4 ml 2-aminoethanol, length of time: 72 hours, washing + 2 ml merkaptoethanol, length of time: 24 hours.

These pollen grains were investigated with the LM and SEM method.

Results

Dry pollen grains (Plate 4.1., fig. 1)

The dry pollen grains are typically spindle shaped, with pointed polar area. The length of the polar axis varies from 22.5 µm to 30.0 µm, maximum (48.0%) at 27.5 µm. P/E ratio from 1.12 to 2.2, maximum (30.5%) at 1.57. The refraction of light is characteristic, and useful to get some information about the inner structure of the pollen grains.

Fresh pollen grains mounted in glycerine-jelly (Plate 4.1., figs. 2,3)

The shape of the pollen grains is more or less elliptical, generally with open sulcus. The proximal surface of pollen is characteristically marked with an area of differentiation which is circular in shape.

Length of the polar axis of the unstained (A) pollen grains from 22.5 µm to 30.0 µm, maximum (68.0%) at 25.0 µm. P/E ratio from 1.12 to 1.9, maximum (35.0%) at 1.8. (Plate 4.1., fig. 2).

Length of the polar axis of the stained (B) pollen grains from 20.0 µm to 27.5 µm, maximum (74.0%) at 25.0 µm. P/E ratio from 1.11 to 1.83, maximum (56.5%) at 1.25 Plate 4.1., fig.3).

Hydrated pollen grains (Plate 4.1., figs. 4,5)

The shape and the apertural area of these pollen grains is similar to the previous one.

Length of the polar axis of the unstained (A) pollen grains from 22.5 µm to 27.5 µm, maximum (83.0%) at 25.0 µm. P/E ratio from 1.11 to 1.9, maximum (38.5%) at 1.8.

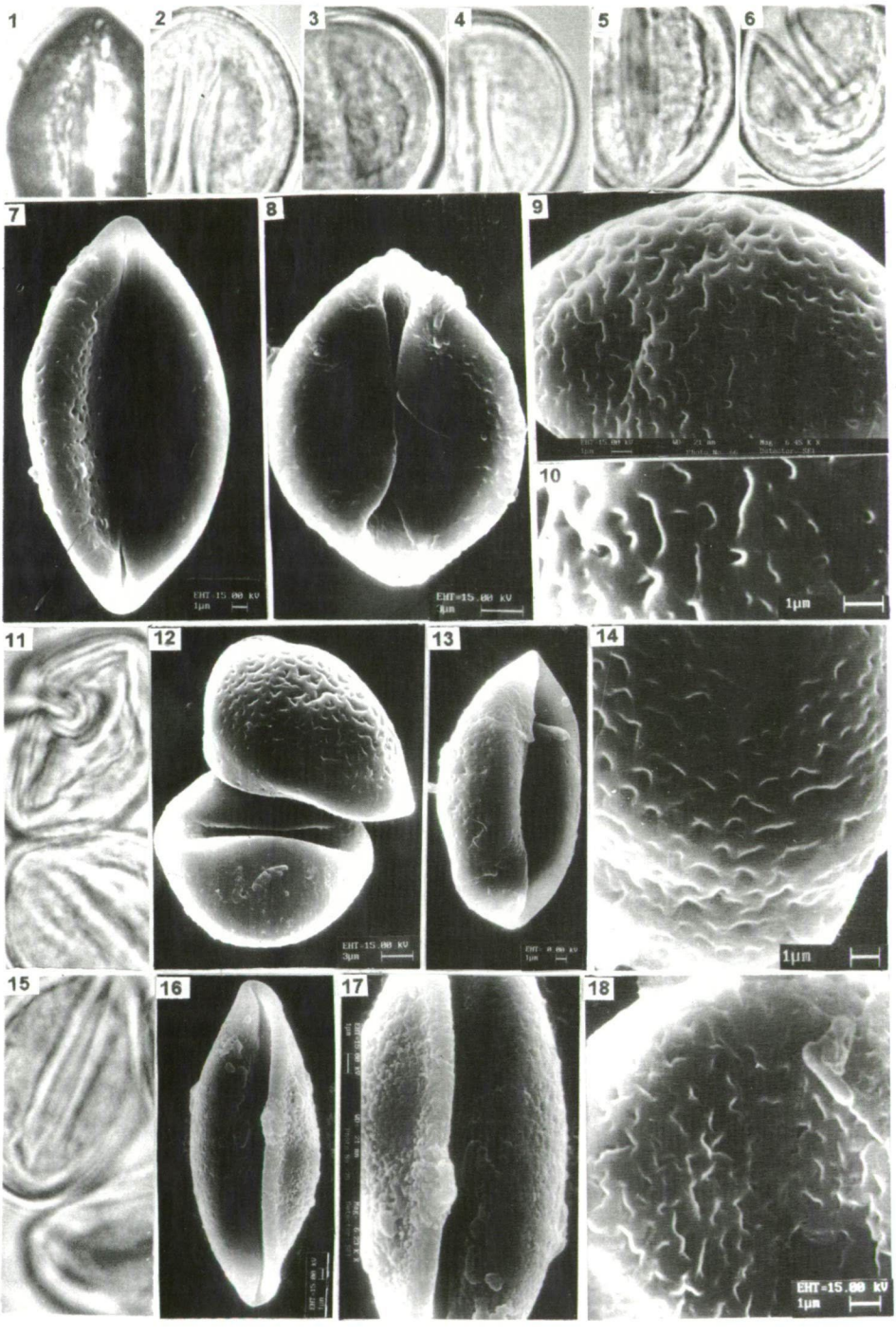

Plate 4.1.

Plate 4.1.

1-18. *Cycas rumphii* MIQ.
1-6. LM pictures 1.650x, 1. Dry pollen grain, 2,3. Fresh pollen grains mounted in glycerine-jelly, 2. Unstained, 3. Stained pollen grains with Methylviolet. 4,5. Hydrated pollen grains, 4. Unstained, 5. Stained
6-10. Partially degraded pollen grains with 2-aminoethanol (24 hours), 6. LM picture.
7-10. SEM pictures, 7,8. General survey picture from the distal side of the pollen grains, 9,10. Detail of the superficial ornamentation of the proximal side.
11-14. Partially degraded pollen grains with 2-aminoethanol (48 hours), 11. LM picture.
12-14. SEM pictures, 12,13. General survey pictures from both sides of the pollen grains. 14. Detail of the superficial ornamentation of the proximal side.
15-18. Partially degraded pollen grains with 2-aminoethanol (72 hours), 15. LM picture. 16-18. SEM pictures, 16,17. General survey pictures from the distal side of the pollen grains, 18. Detail of the sculpture of the proximal side.

Length of the polar axis of the stained (B) pollen grains from 22.5 µm to 27.5 µm, maximum (80.5%) at 25.0 µm. P/E ratio from 1.12 to 1.9 maximum (41.0%) at 1.8.

Partial degradation with 2-aminoethanol (Plate 4.1., figs. 6-18)

Partial degradation (24 hours). experiment No: T-12-148 (Plate 4.1., figs. 6-10)

LM results (Plate 4.1., fig. 6). The alteration of the pollen grains mounted in glycerine-jelly is similar to the previous experiments. Length of the polar axis: from 22.5 µm to 27.5 µm, maximum (81.5%) at 25.0 µm. P/E ratio from 1.11 to 1.57, maximum (52.0%) at 1.25.

SEM results (Plate 4.1., figs. 7-10). The shape of the pollen grains is similar to the dry and fresh pollen grains (Plate 4.1, figs. 7,8). The early sulcus type is well shown in picture 8, of the Plate 4.1.). The characteristic rugulate sculpture of the surface is well illustrated in the highly magnified SEM photographs (Plate 4.1, figs. 9,10).

Partial degradation (48 hours), experiment No.: T-12-149 (Plate 4.1., figs. 11-14)

LM results (Plate 4.1., fig. 11). Degradation of the pollen wall was not detectable with the light microscope. The shape of the pollen grains ellipsoidal, length of the polar axis from 22.5 µm to 27.5 µm, maximum (74.5%) at 25.0 µm. P/E ratio from 1.11 to 1.66, maximum (47.0%) at 1.25.

SEM results (Plate 4.1., figs. 12-14). Changes in morphological features are well illustrated in SEM photographs (Plate 4.1., figs. 12,13). On the distal face of the pollen superficial degradation may be noticed.The characteristic rugulate sculpture may be observed on the proximal surface of one pollen grain (Plate 4.1., fig. 12) and in the highly magnified one (Plate 4.1., fig. 14).

Partial degradation (72 hours), experiment No.: T-12-150 (Plate 4.1., figs. 15-18)

LM results (Plate 4.1., fig. 15). The shape of the pollen grains is unchanged in contrast to the previous one, but a superficial degradation may be observed, the surface seems a little granular. Polar axis from 22.5 µm to 27.5 µm, maximum (78.5%) at 25.0 µm. P/E ratio from 1.11 to 166, maximum (49.0%) at 1.25.

SEM results (Plate 4.1., figs. 16-18). A general survey reveals that the superficial ornamentation is more characteristic (Plate 4.1., figs. 16,17), in all probablity in consequence to the degradation process. High magnification (Plate 4.1., fig. 18) illustrates well the characteristic sculpture.

Partial degradation with 2-aminoethanol and potassium permanganate (Plate 4.2.-4.)

Experiment No: T-12-151 (Plate 4.2., figs. 1-10)

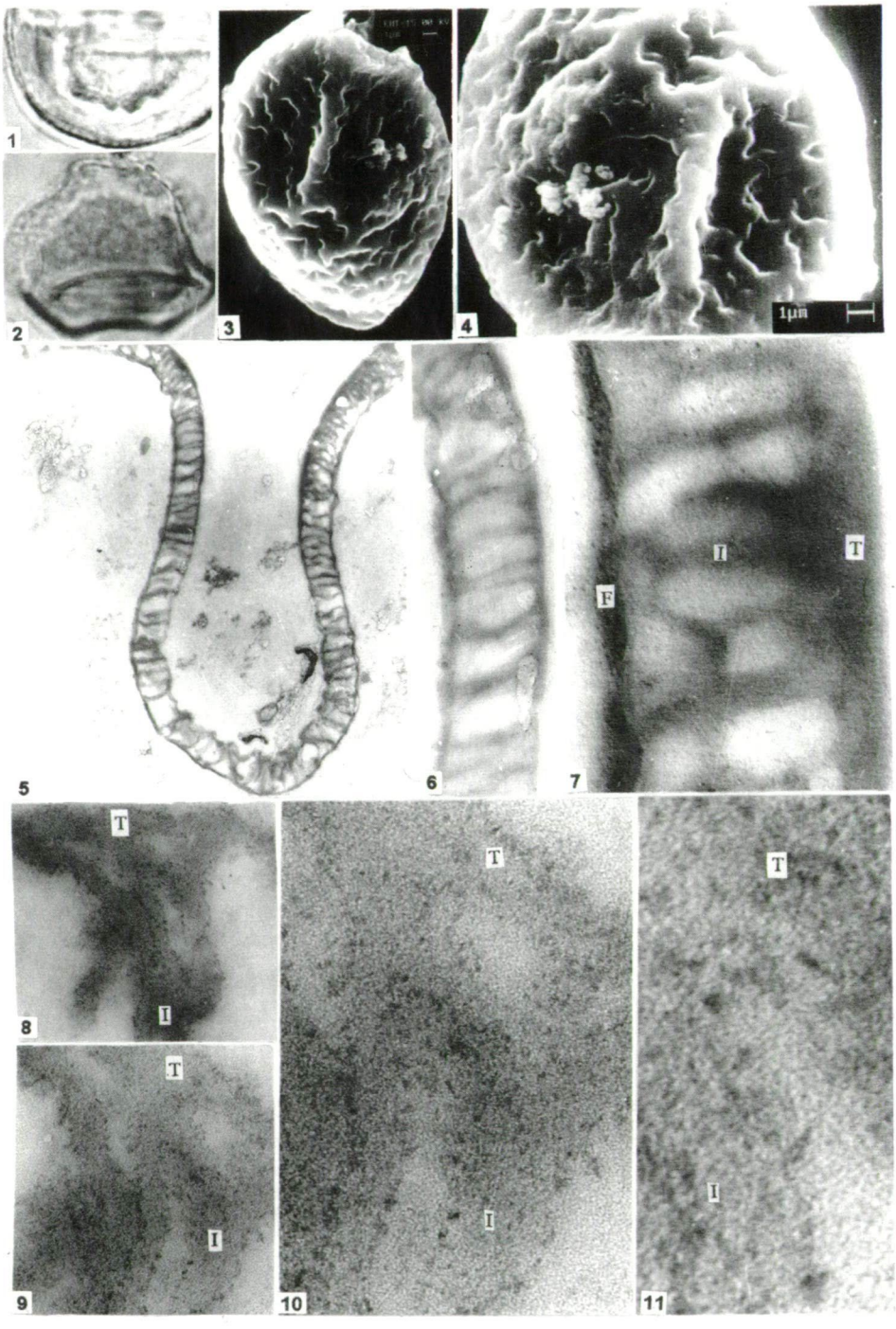

Plate 4.2.

Plate 4.2.

1-11. *Cycas rumphii* MIQ. Partially degraded pollen grains with 2-aminoethanol (24 hours) and $KMnO_4$ (24 hours).

1.2. LM pictures, 1.650x. 1. Pollen grain mounted in glycerine-jelly after treatment, 2. Pollen grain after experiment and embedding processes, mounted in Araldite. The degradation effect of the embedding processes is well illustrated.

3,4. SEM pictures. The degradation of the superficial ornamentation is well shown.

5-11. TEM pictures. 5. General survey picture of the ectexine ultrastructure in the apertural area. Negative No.: 8821, 9.910x, 6,7. Details of the ectexine ultrastructure, 6. Negative No.: 8823, 33.035x, 7. The electron density of the partially degraded foot layer is well shown. Negative No.: 8824, 99.107x, 8-11. Biopolymer structure of the partially degraded ectexine, 8. Negative No.: 10712, 99.107x, 9. Negative No.: 10713, 165.178x, 10. Negative No.: 10714, 330.357x, 11. Negative No.: 10715, 660.714x.

LM results (Plate 4.2., figs. 1,2) Pollen grains mounted in glycerine-jelly after the experiment appear not so degraded (Plate 4.2., fig. 1). Polar axis from 20.0 µm to 27.5 µm, maximum (64.5%) at 25.0 µm. P/E ratio from 1.11 to 1.66, maximum (39.0%) at 1.25. But after the embedding process important alterations may be observed with the light microscope also (Plate 4.2, fig. 2). The degradation of the ectexine is evident, the intine is swollen, the electron dense protoplasm is contracted. Polar axis from 17.5 µm to 25.0 µm, maximum (48.0%) at 22.5 µm. P/E ratio from 1.11 to 2.0, maximum (25.5%) at 1.14.

SEM results (Plate 4.2., figs. 3,4) The characteristic superficial ornamentation has not changed appreciably, but the superficial degradation is remarkable.

TEM results (Plate 4.2., figs. 5-11)

Ultrastructure of the investigated pollen (Plate 4.2., fig. 5) clearly illustrates the thick tectum, foot layer and the alveolar infratectal layer. The intine is degraded and some dark remnants indicate the presence of this layer. In highly magnified photographs of the ectexine (Plate 4.2., figs. 6,7) the radially oriented alveolar system of the infratectal layer is better shown. Electron dense foot layer and degradation of the ectexine was clearly observed (Plate 4.2., fig. 7). Photographs taken at high resolution EM (Plate 4.2., figs. 8-10) illustrate well the degradation process in biopolymer at molecular level. The globular biopolymer units are arranged in the inner surfaces of the alveolar infratectal units (cf. Plate 4.2., figs. 8-10). The electron dense globular units are 5-8 Å in diameter. It is worth mentioning that quasi-crystalloid biopolymer units were not observed.

Experiment: T-12-152 (Plate 4.3., figs. 1-10)

LM results (Plate 4.3., figs. 1,2) Pollen grains mounted in glycerine-jelly after partial degradation seem not to be damaged. The shape is a little spindel like. Polar axis from 20.0 µm to 27.5 µm, maximum (71.5%) at 25.0 µm. P/E ratio from 1.11 to 1.66, maximum (46.0%) at 1.25. After embedding processes the pollen grains mounted in Araldite (Plate 4.3., fig. 2) are extremely damaged. Degradation of the ectexine and the intine is apparent. The electron dense protoplasm seems to be compact in the light microscope. Polar axis from 17.5 µm to 30.0 µm, maximum (57.5%) at 22.5 µm. P/E ratio from 1.0 to 1.9, maximum (42.5%) at 1.12.

SEM results (Plate 4.3., figs. 3,4) The general survey illustrates well the experimentally damaged pollen grains (Plate 4.3., fig. 3). In high magnification (Plate 4.3., fig. 4) difference in ornementation of the two surfaces are clear and original sculpture has essentially remained unchanged.

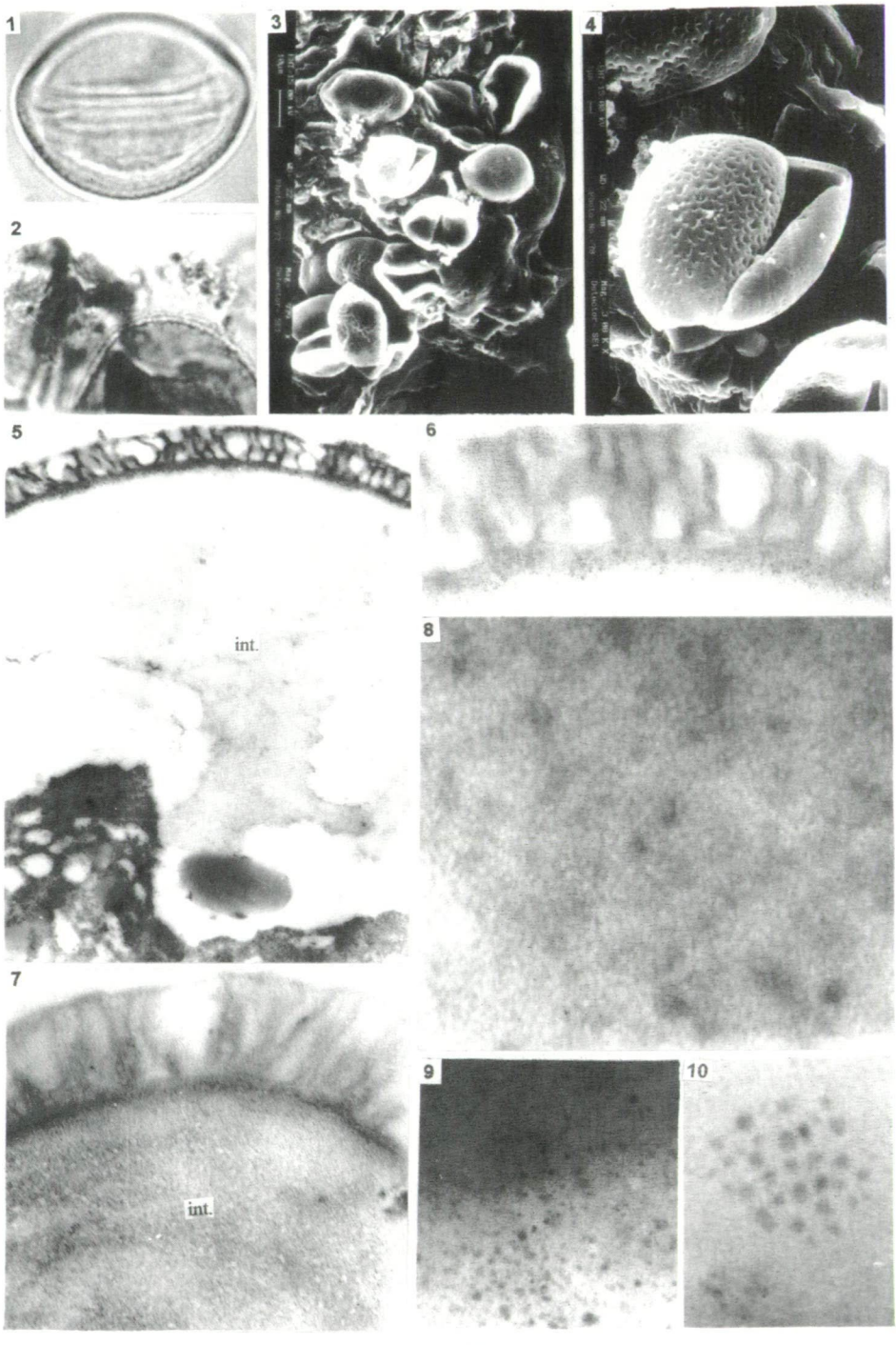

Plate 4.3.

Plate 4.3.

1-10. *Cycas rumphii* MIQ. Partially degraded pollen grains with 2-aminoethanol (48 hours), and $KMnO_4$ (24 hours).

1,2. LM pictures, 1.650x, 1. Pollen grains mounted in glycerine-jelly after treatment, 2. Pollen grains after experiment and embedding processes mounted in Araldite.

3,4. SEM pictures. 3. General survey picture of the partially degraded pollen grains, 4. A degraded pollen grain in high magnification.

5-10. TEM pictures, 5. General survey picture of the ectexine, intine and the other part of the protoplasm. Negative No.: 8797, 9.910x, 6. Detail from the partially degraded ectexine. The degradation of the foot layer is characteristic, Negative No.: 8799, 7. Details from the partially degraded pollen wall. Illustrated are the damaged ectexine and intine. Remnants of the lamellar ultrastructure of the intine are well shown. Negative No.: 8801, 33.035x, 8. Molecular system and highly organized globular biopolymer units of the partially degraded ectexine. Negative No.:10629, 660.714x, 9. Biopolymer structure at different degradation level. Negative No.: 10627, 165.178x, 10. Cluster of globular biopolymer units from the partially damaged infratectal layer. Negative No.: 10629, 330.357x.

TEM results (Plate 4.3., figs. 5-10) The extremely thick and partially degraded intine, the plasma membrane and protoplasm is well illustrated (Plate 4.3., fig. 5). Degradation of foot layer was noticed (Plate 4.3., fig. 6). Remnants of damaged lamellar ultrastructure of probable ectintine is illustrated in fig. 7 of Plate 4.3. The high resolution TEM photographs clearly show the damaged globular biopolymer structure and the extremely complicated molecular structure of the ectexine (Plate 4.3., figs. 8-10). An interesting and peculiar cluster of globular biopolymer unit was observed in the degraded alveolar infratectal layer (Plate 4.3., fig. 10). Different kinds of molecular arrangements such as - hexagons, regular pentagon, and tetragons can be identified. This is new obseravation in comparison to our previous results.

Experiment No.: T-12-153 (Plate 4.4., figs. 1-9)

LM results (Plate 4.4, figs. 1,2). Pollen grains after experiment mounted in glycerine-jelly are not damaged much (Plate 4.4., fig. 1). Polar axis from 20.0 µm to 27.5 µm, maximum (58.5%) at 25.0 µm. P/E ratio from 1.12 to 1.09, maximum (28.0%) at 1.8. After the embedding processes damage in ectexine is evident (Plate 4.4., fig. 2). The intine thickened, and the protoplasm shrinked seemingly becoming electron dense. The original morphological features observed in light microscope may not be observed in the deformed pollen grains. Polar axis from 17.5 µm to 25.0 µm, maximum (42.0%) at 22.5 µm. P/E ratio from 1.11 to 1.8, maximum (20.0%) at 1.14.

SEM results (Plate 4.4., figs. 3,4). The characteristic shape of the pollen grains and the aperture is well illustrated. Disintegration of the ectexine is clearly visible.

TEM results (Plate 4.4., figs. 5-9). Electron dense protoplasm and other organelles may be observed (Plate 4.4., figds. 5,6). Damaged and deformed pollen were noticed (Plate 4.4., figs. 5,8). Degradation of the ectexine is also clear (Plate 4.4., fig. 7). The foot layer is electron dense and an inner layer, probably ectintine, may be observed. High resolution photographs illustrate strongly damaged biopolymer structure (Plate 4.4., fig. 9). The highly organized globular molecular units disappeared, and different kinds of molecular systems (cyclic, linear) were noticed.

Partial degradation with 2-aminoethanol, and merkaptoethanol (Plate 4.5., figs. 1-10)

Experiment No.: T-12-154 (Plate 4.5., figs. 1-3)

LM results (Plate 4.5., fig.1). No significant alterations in morphological features of the pollen grains were noticed. Polar axis from 20.0 µm to 30.0 µm, maximum (70.0%) at 25.0 µm. P/E ratio from 1.0 to 2.0, maximum (26.0%) at 1.25.

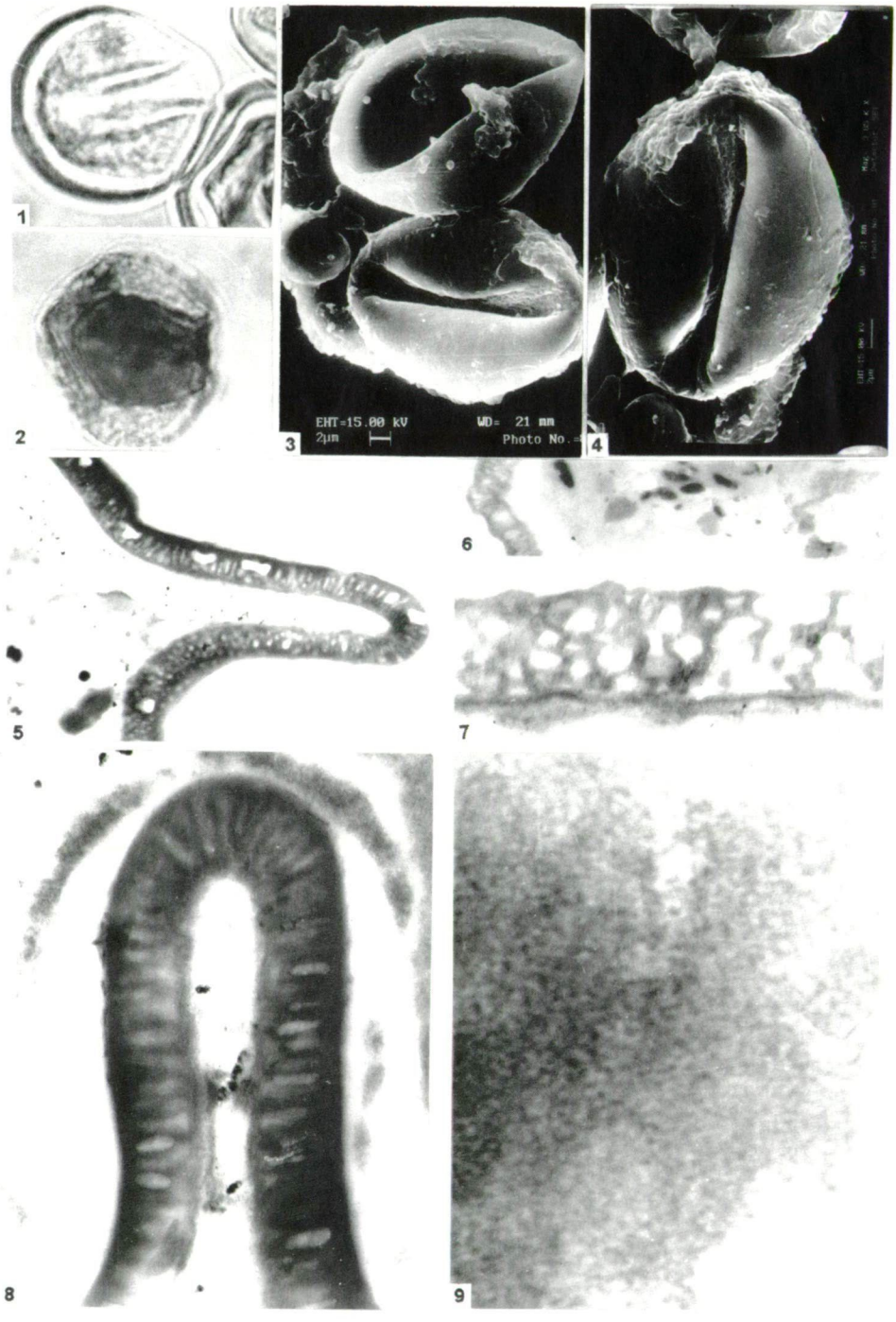

Plate 4.4.

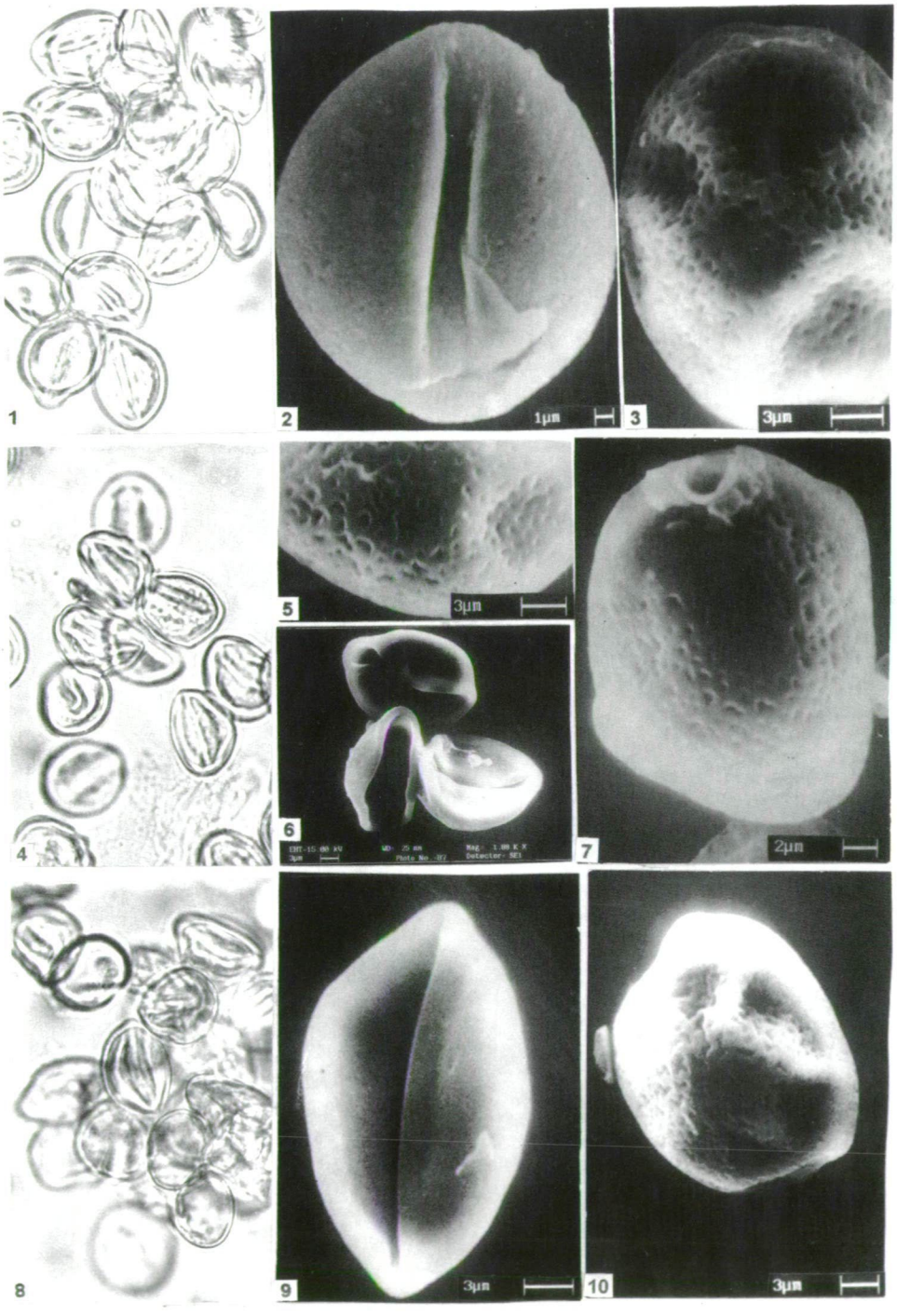

Plate 4.5.

Plate 4.4.

1-9. *Cycas rumphii* MIQ. Partially degraded pollen grains with 2-aminoethanol (72 hours) and $KMnO_4$ (24 hours)
1,2. LM pictures, 1.650x, 1. Pollen grains mounted in glycerine-jelly after treatment, 2. Pollen grains after experiment and embedding processes, mounted in Araldite.
3,4. SEM pictures of the distal side of the pollen grains.
5-9. TEM pictures, 5,6. Ultrastructure of the ectexine and the damaged intine. 5. Negative No.: 8806, 9.910x, 6. Negative No.: 8811, 9.910x, 7. Detail from the partially degraded ectexine. Under the foot layer a damaged endexine may be presumed. Negative No.: 8701,, 33.035x, 8. Detail from the ectexine of a damaged and deformed pollen grain. Negative No.: 8807, 33.035x, 9. Molecular system of the partially degraded tectum and infratectal layer. The highly organized globular biopolymer units are not perceptibles, as they were distroyed. Negative No.: 10617, 660.714x.

Plate 4.5.

1-10. *Cycas rumphii* MIQ.
1-3. Partially degraded pollen grains with 2-aminoethanol (24 hours) and merkaptoethanol (24 hours).
1. LM picture, 660x.
2,3. SEM pictures, 2. Distal surface with the apertural area, 3. Proximal side of the pollen grains, the foveolate ornamentation is well shown.
4-7. Partially degraded pollen grains with 2-aminoethanol (48 hours) and merkaptoethanol (24 hours).
4. LM picture, 660x
5-7. SEM pictures, 5,7. Detail from the fine sculpture of the proximal surface, 6. General survey picture of three pollen grains in different position.
8-10. Partially degraded pollen grains with 2-aminoethanol (72 hours) and merkaptoethanol (24 hours).
8. LM picture, 660x.
9,10. SEM pictures, 9. Distal side of the pollen grain, 10. Proximal superficial ornamentation of the pollen grain.

SEM results (Plate 4.5., figs. 2,3). In contrast to the previous results, the surface ornamentation is foveolate, or sometimes reticulate. This is the superficial view of the perforated tectum.

Experiment No.: T-12-155 (Plate 4.5., figs. 4-7)

LM results (Plate 4.5., fig. 4).The basic morphology of the pollen grains is similar to the previous experiment. Polar axis from 22.5 µm to 27.5 µm, maximum (72.0%) at 25.0 µm. P/E ratio from 1.11 to 1.83, maximum (35.0%) at 1.25.

SEM results (Plate 4.5., figs 5-7). The basic morphology of the pollen grains is illustrated in picture 6, Plate 4.5. The surface ornamentation is also foveolate (Plate 4.5., figs. 5,7).

Experiment No.: T-12-156 (Plate 4.5., figs. 8-10).

LM results (Plate 4.5, fig. 8). Similar to the previous ones. Polar axis from 20.0 µm to 30.0 µm, maximum (72.5%) at 25.0 µm. P/E ratio from 1.0 to 2.0, maximum (28.0%) at 1.11.

SEM results (Plate 4.5., figs. 9,10). No important differences were observed in contrast to the previous experiments.

Discussion and Conclusions

1.Light microscopic studies of the dry pollen with the help of refrection of light have been attempted earlier (KEDVES, BORSODI, DOBÓ, KÓVÁCS and SZÉCSÉNYI, 1999a,b). In the present contribution we have limited our light refraction observations to study the shape of pollen which are spindle shaped only.

2. Alterations in consequence to the hydratation are important because the sedimentation of the sporomorphs start in water condition. Studies in this respect have already been carried out.

3. The monting media (glycerine-jelly) and the staining may also modify the morphology of the pollen grains. Our results concerning the P/E ratio may be summarized as follows:

3.1. In the dry pollen grains the maximum (30.5%) was at 1.57. This value in the experimental material is very different.

3.2. In fresh pollen grains mounted in glycerine-jelly differences were noticed between the unstained and stained pollen grains.

3.3. Similarities were observed as follows:

3.3.1. Hydrated pollen grains (A and B), unstained (A) fresh and unstained fresh and unstained pollen grains treated with 2-aminoethanol (72 hours) and $KMnO_4$ (24 hours).

3.3.2. All unstained pollen grains degraded with 2-aminoethanol during 24, 48 and 72 hours, pollen grains treated with 2-aminoethanol (24 hours) and $KMnO_4$ (24 hours).

Stained fresh pollen grains mounted in glycerine-jelly.

Unstained pollen grains treated with 2-aminoethanol during 24 and 48 hours and $KMnO_4$ (24 hours).

Unstained pollen grains treated with 2-aminoethanol during 24 and 48 hours and $KMnO_4$ (24 hours).

3.3.3. All experimental pollen grains mounted in Araldite and degraded with 2-aminoethanol (72 hours) and merkaptoethanol (24 hours).

3.4. As regards the maximum of the polar axis three groups were distinguished: 1. The dry pollen grains, 2. Experimental pollen grains mounted in Araldite. 3. All others (fresh, hydrated and a great part of the experimental pollen grains).

4. We wanted to observe the morphological alterations, if any, that took place during the preparation of the pollen grains for transmission electron microscopy. The light microscopic observations in embedded pollen grains in comparison to the partially degraded pollen grains mounted in glycerine-jelly illustrate well the corrosion during embedding processes caused by OsO_4 aq. dil. (cf. FREDERIKSEN, 1976). The "inner body" (AMBWANI and KUMAR, 1991) is relatively resistant.

5. Pollen grains partially degraded with 2-aminoethanol are foveolate and finely rugulate. Oxidation with $KMnO_4$ resulted in disappearance of the characteristic rugulate surface. Interestingly, partial degradation with 2-aminoethanol and merkaptoethanol resulted in fine foveolate surface.

6. The ultrastructure of the partially degraded pollen grains is also interesting. The foot layer of the ectexine was firstly damaged. Peculiarities observed in the biopolymer organization of the ectexine may be summarized as follows:

6.1. During our investigations we have not observed any quasi-crystalloid or quasi-equivalent biopolymer structures.

6.2. Electron dense globular biopolymer structures were seen at the surfaces of the alveolar infratectal layer after moderate degradation processes (Plate 4.2., figs. 10,11).

6.3. An unusual and peculiar cluster of electron dense globular biopolymer units was observed at the base of the infratectal layer after strong degradation. This unit may represent tetragons, regular pentagons (quasi-crystalloid element) and hexagon (element of the quasi-equivalent biopolymer system).

6.4. High resolution photographs illustrate the molecular structure also which seems to be very complex, cyclic or in chain.

Finally, further experiments with C60 fullerene/benzol solution are in progress.

Acknowledgements

Authors thank the authorities at Indian National Science Academy, New Delhi and the Hungarian Academy of Sciences, Budapest for cooperation which made it possible to undertake this joint work. We also sincerely acknowledge the encouragement and cooperation received from Prof. A.K. SINHA, Director, Birbal Sahni Institute of Palaeobotany, Lucknow, India. This work was supported by Grant OTKA T 31715.

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5. BIOPOLYMER STRUCTURE OF THE PARTIALLY DEGRADED CUTICLES OF CYCAS RUMPHII MIQ.: A PRELIMINARY REPORT

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During our previous research programs we carried out symmetry operations on the biopolymer structure of partially degraded pollen grains and the wall of *Botryococcus braunii* KÜTZ isolated from Hungarian oil shale. Under a new joint research program investigations are in progress on the pollen grains and leaf cuticles of *Cycas rumphii* MIQ. collected from the garden of the Birbal Sahni Institute of Palaeobotany. After partial degradation of these materials with different methods, investigations were made by LM, SEM and TEM. Results of these studies on pollen grains are presented in this number (TRIPATHI, MADHAV KUMAR, KEDVES and VARGA, 2003).

Sequel to partial degradation of cuticles the biopolymer structure was also discovered. Transmission Electron Microscopic studies revealed several kinds of biopolymer organization. In this preliminary report a new and unexpected result, the presence of the quasi-crystalloid biopolymer network in the leaf cuticle of *Cycas rumphii* (Experiment: T-12-162. - 2 mg mature leaf + 2 ml 2-aminoethanol, duration: 48 hours, washing, + 10 ml $KMnO_4$ 1% for 24 hours, at 30 °C temperature) has been communicated. A negative regular pentagon was observed for the first time (Plate 5.1., figs 1,2). We used the fivefold, and the tenfold rotation (Plate 5.2., figs. 1-3). This method verified the presence of quasi- crystalloid skeleton in the leaf cuticles. Concerning the fivefold rotation we have given the rotation picture also which was not made from the centre of the fivefold biopolymer unit. This is the second time when we have presented such a picture but these deficient rotation pictures provide interesting informations about the organization of biopolymer system.

Negative quasi-crystalloid biopolymer network was first observed in the partially degraded exospore of *Equisetum arvense* L. (KEDVES and PÁRDUTZ, 1993). Later, KEDVES, PÁRDUTZ, TÈRBE and HORVÁTH (2001) made rotation in negative regular pentagon unit observed in partially degraded ectexine of *Encephalartos ferox* BERTOL.

In this preliminary report we would like to point out the following:

1. The history of the chemistry of the resistant biopolymer of the pollen wall started with the paper of JOHN (1814). This was followed by several publications among which reports by ZETZSCHE and his coworkers are important (ZETZSCHE and VICARI, 1931, ZETZSCHE et al., 1931). ZETZSCHE and HUGGLER (1928) for the first time established the polyterpene structure for the sporin. In a comprehensive paper TOMSOVIC (1960) pointed out that the sporopollenin is a highly polymerized terpene derivative similar to cutin. The British School (BROOKS and SHAW, 1968,1972) suggested that sporopollenin

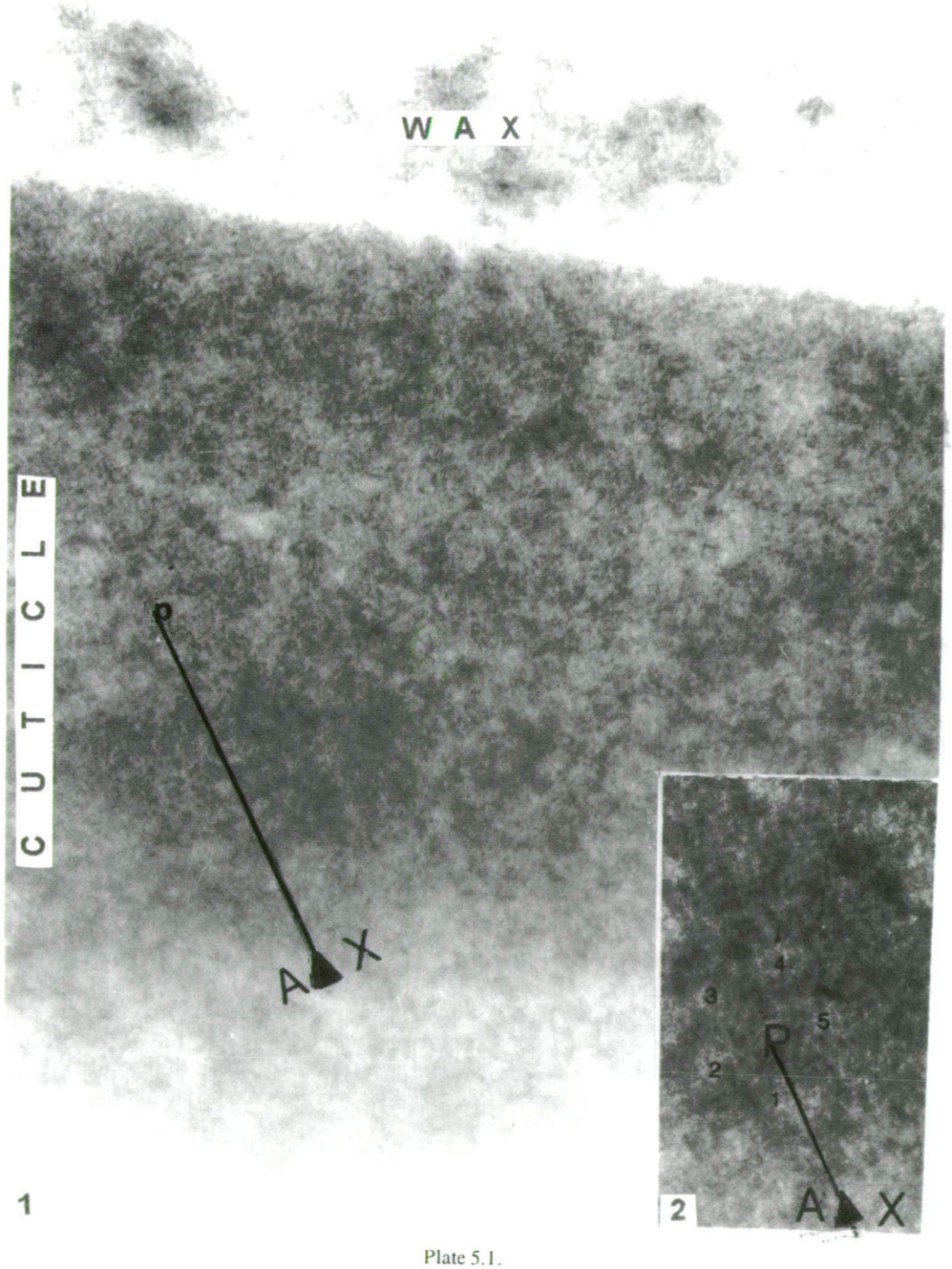
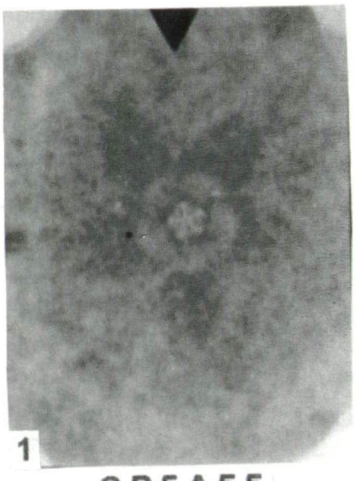


Plate 5.1.



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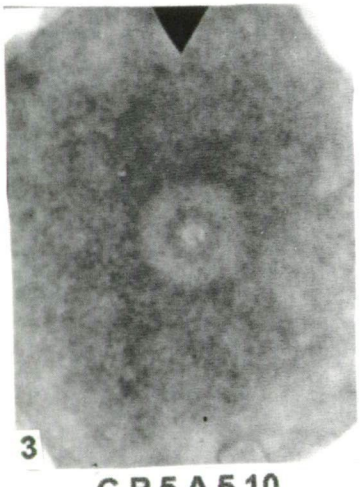
C.P.5.A.5.5.



C.P.5.A.5.10.

Plate 5.2.

Plate 5.1.

Cycas rumphii MIQ. Ultrastructure of partially degraded cuticle.1. General survey picture of the wax and the cuticle, 200.000x. 2. Negative regular pentagon of the partially degraded cuticle 500.000x.

Plate 5.2.

Primary rotations pictures of the negative regular pentagon. 250.000x.

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is a biopolymer of βcarotene and its oxidized esters. With the help of extensive investigations the sporopollenin was found to occur in the pollen walls of higher plants, some algae and fungi, in the walls of fossil *Tasmanites* and Carboniferous megaspores. Further studies later verified the complexity in biopolymer structure of the plant cell walls.

2. After the period, which may be marked as the time of general characterization and occurrence of the sporopollenin, based on the new data several categories were identified for the substances which constituted the plant cell walls. The term algaenan was used by LARGEAU et al. (1986), KADOURI et al. (1988), TEGELAAR et al. (1989), DE LEEUW, VAN BERGEN et al. (1991) for some algal walls, botryococcene for the highly unsaturated isoprenoid hydrocarbons (DUBREUIL, DERENNE, LARGEAU et al., 1989) and botryococcane for the fossil biopolymer (DERENNE, LARGEAU, CASADEVALL and CONNAN, 1988a,b, DUBREUIL, DERENNE, LARGEAU et al., 1989, BRENNER, 1998).

3. Several papers concerning chemistry of leaf cuticle have been published. KOLATTUKUDY and ESPELIE (1985), MARTIN and JUNIPER (1970), RIEDERER (1991) VAN BERGEN, SCOTT, BARRIE, DE LEEUW and COLLINSON (1994) opined that they are composed of a vax fraction, soluble in organic solvents and an insoluble matrix. This matrix consists either of the biopolyester cutin or of an insoluble, non-hydrolyzable biomacromolecule, recently named as cutan (TEGELAAR et al., 1989) or most commonly, a mixture of both.

The composition of biopolymer systems of plant cell walls are thus different. Several types were identified within the so-called sporopollenin and also in the biopolymers. The basic structure of the biopolymer is composed by the highly organized molecules of different levels. Biopolymer units of angstrom and nanometer dimensions have already been established (KEDVES 1989). It appears that inspite of differences in the chemical nature of plant cell walls there exist analogies or similarities at the organization level.

The quasi-crystalloid system is well established in the ectexines of different taxa of gymnosperms and angiosperms, in the botryococcane and now we have demonstrated it in the cutin for the first time, by in vitro method.

This work was supported by Grant OTKA T 31715. S.K.M. TRIPATHI wishes to thank the authorities of Indian National Science Academy, New Delhi and the Hungarian Academy of Sciences, Budapest for cooperation because these works were finalised during his visit to Szeged under the Exchange of Scientists Programme. He is also thankful to Prof. A.K. SINHA, Director, Birbal Sahni Institute of Palaeobotany for his kind cooperation and encouragement throughout the collaborative researches between BSIP and CBEM Lab, Szeged, Hungary.

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6. TRANSMISSION ELECTRON MICROSCOPY OF THE CONNECTIVES OF THE POLLEN GRAINS OF GINKGO BILOBA L.

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Short communication

There are several publications concerning the pollen grains of *Ginkgo biloba* L. LM data of recent and fossil pollen grains was reviewed in 1961 (KEDVES). Preservation of cycad and *Ginkgo* pollen was investigated by FREDERIKSEN (1978). TEM data by UENO (1960), AUDRAN and MASURE (1975, 1978 in this latter paper the differences between the exine ultrastructure of *Ginkgo biloba* and the Cycadales were emphasized), Further TEM data by XI YI ZHEN and WANG FU-HSIUNG (1989), XI YI-ZHEN (1990). KEDVES and PÁRDUTZ (1997) investigated the alterations in the ultrastructure of the pollen grains of *Ginkgo biloba* in consequence of the X-ray irradiation. During our experimental studies on the high temperature etc. effect on gymnosperm pollen grains (KEDVES, TÓTH and FARKAS, 1991) peculiar connectives were observed between two pollen grains of *Ginkgo biloba* L. This feature was observed in 1% of the pollen grains, and is believed to be an early characteristic. Stained with Toluidine blue the so-called pore or pseudopore was violet coloured, the other parts of the ectexine are greenish-blue. The stain acceptance indicated differences in the molecular structure of the different parts of the exine.

During one of the programs of our laboratory, different kinds of partial degradation were carried out on the pollen grains of this species too, and a great number of TEM pictures were made. At a very few ultrathin sections of two experiments (T-12-224 and T-12-225) we observed of these peculiar connectives. Our up to date results following the different experiments may be summarized as follows: No important differences were found between the ultrastructure in the two experiments from this point of view.

Fig. 1 (Plate 6.1.) illustrate well the ultrastructure of the inter-apertural and the apertural area, and the extremely swollen intine. The reduction of the tectum and the infratectal layer is well shown. The ultrastructure of the connectives may be summarized as follows: The tectum around the pseudopores thickened in an important measure, approximaltely twofold. (Plate 6.1., fig. 2). The elements of the infratectal layer are also longer than in the other part of the inter-apertural area. No alterations were observed in the ultrastructure of the foot layer and the intine (Plate 6.1., figs. 1-3). In the middle of the connectives there is a depression of the tectum or a complete disappearance of this layer (Plate 6.1-3.).

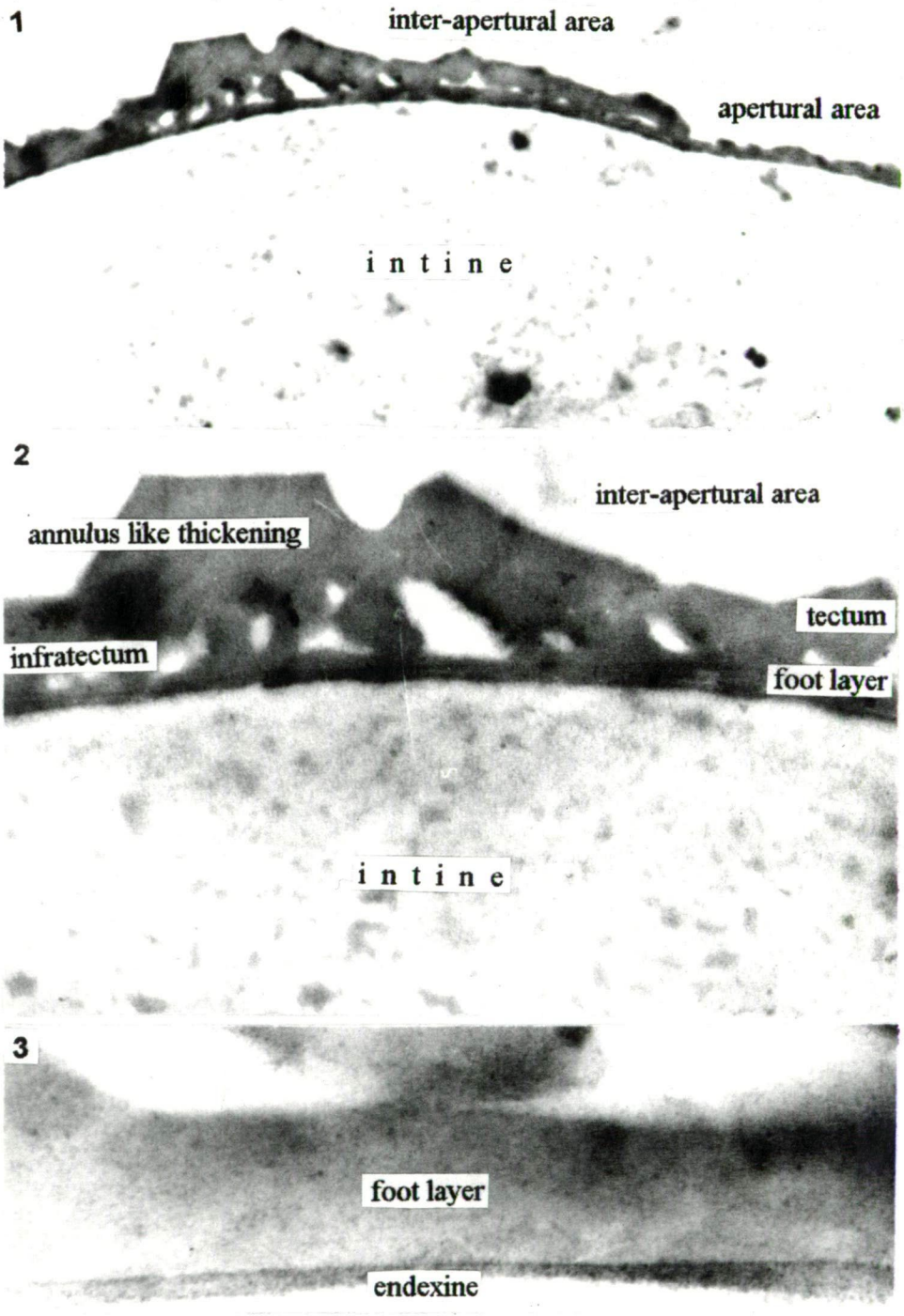

Plate 6.1.

Plate 6.1.

1- 3. *Ginkgo biloba* L. Ultrastructure of partially degraded pollen grains
1,2. Experiment: T-12-224 (5 mg dry pollen + 2 ml 2-aminoethanol, length of time: 48 hours, washing, + 2 ml merkaptoethanol. Temperature: 30°C). 1. 10.000x, Negative No.: 9468, 2. 25.000x, Negative No.: 9470.
3. Experiment: T-12-225 (5 mg dry pollen + 2 aminoethanol, length of time: 72 hours, washing, + 2 ml merkaptoethanol Temperature: 30°C). 100.000x, Negative No.: 9510.

In short we observed for the first time the ultrastructure of this really extremely scarce morphological characteristic feature of this early pollen type.

This work was supported by Grant OTKA T 31715, and PRCH Student Science Foundation, DT 2001. nov./2.. We express our sincere thanks to Eric CAULTON (Director, Scottish Centre for Pollen Studies, Edinburgh, Scotland, UK) reviewing the manuscript and for his valuable comments.

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7. EFFECT OF THE HIGH TEMPERATURE AND THE C60 FULLERENE/ BENZOL SOLUTION TO THE POLLEN GRAINS OF GINKGO BILOBA L. AND QUERCUS ROBUR L.

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Abstract

Two types of experiments were carried out on pollen grains of *Ginkgo biloba* L. and *Quercus robur* L.: 1. The first experiment noted the affect of a 30 °C temperature on these pollen grains for different length of time: 10 minutes, 1, 5, and 10 hours. The second experiment noted the effect of C60 fullerene/benzol solution on the LM morphology and ultrastructure of the pollen grains.

Key words: Experimental Palynology, recent, LM, TEM.

Introduction

It has been a considerable time that our Laboratory has investigated the effect of high temperature on different kinds of pollen and spores. In the past the pollen spores were subjected to temperatures of 100 °C and usually 200 °C. When subjected to these temperatures important morphological alterations were observed in the studied pollen grains (KEDVES and KINCSEK, 1989, KEDVES, TÓTH and FARKAS, 1991). In view of these results, we decided to investigate the effect of 30 °C temperatures on selected pollen grains inasmuch as these lower temperatures may also have an affect occurring in nature during the dispersion of the pollen before sedimentation.

The study of the biopolymer structure and symmetry is another program currently undertaken in our Laboratory. Different kinds of solvents and oxidizing agents are being used to observe the partial degradation of the sporoderm. Initially, the application of C60 fullerene/benzol solution was used to observe sporoderm degradation (KEDVES and FREY, 2002). Later, the application of the above techniques on *Taxus baccata* pollen also were succesful (KEDVES, PÁRDUTZ, JACSÓ and KOCSICSKA, 2002). The biopolymer symmetry studies of KEDVES, BÉRES, JACSÓ and KOCSICSKA (2002) are presented in this volume.

The initial data on the effect of a 30 °C temperature and the results of the C60 fullerene/benzol solution on the fine structure of the two above mentioned pollen grain species are presented herein. The purpose of these studies, presented herein, is to establish the morphological alterations effects when the pollen of the two above mentioned species are subjected to a temperature of 30 °C and also the changes in the ultrastructure when these two pollen species are subjected to C60 fullerene/benzol solution.

Materials and Methods

Ginkgo biloba L. pollen grains was collected by K. PRISKIN (Szeged, cultivated, date: 15. 04.2001), *Quercus robur* L. by B. VARGA (Botanical Garden of the University of Szeged, date: 12.04.2001).

High temperature experiments, on 30 °C

Ginkgo biloba L. T-12-289, dry, fresh pollen grains, T-12-290, heated for 10 minutes, T-12-291, length of time: 1 hour, T-12-292, 5 hours, T-12-293, 10 hours.

Quercus robur L. T-12-284, dry fresh pollen grains, T-12-285, heated for 10 minutes, T-12-286, length of time: 1 hour, T-12-287, 5 hours, T-12-288, 10 hours.

T-12-306. - *Ginkgo biloba* L. 3 mg pollen grains + 5 ml C60 fullerene/benzol solution + 5 ml benzol, temperature 30 °C, length of time: 1 day T-12-307, 2 days, T-12-308, 3 days, T-12-309, 4 days, T-12-310, 5 days, T-12-311, : 6 days. For LM studies unstained, and Methylviolet stained pollen grains were investigated.

T-12-300. - *Quercus robur* L. 3 mg pollen grains + 5 ml C60 fulerene/benzol solution + 5 ml benzol, temperature 30 C, length of time: 1 day, T-12-3001, 2 days, T-12-302, 3 days, T-12-303, 4 days, T-12-304, 5 days, T-12-305, 6 days.

In this paper we present the results of the LM investigations of these pollen grains, and as a preliminary report the TEM data of the first experiments (T-12-306, and T-12-300).

Results

LM results

Ginkgo biloba L. (Plate 7.1., figs. 1-17)

P/E ratio

Experiment N°	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	
T-12-289		8.0	17.5	23.5	23.5	9.5	9.5	5.5	2.0		0.5		0.5	%
T-12-290		8.5	28.5	20.0	16.0	11.5	9.0	4.0	0.5	1.0	0.5		0.5	
T-12-291		1.0	25.5	16.5	21.5	10.5	8.0	5.5	0.5	1.5	0.5			
T-12-292	4.5	20.0	31.0	13.5	15.0	8.0	4.0	2.5	1.0		0.5			
T-12-293	3.0	14.5	22.5	28.0	14.0	13.0	1.0	1.5	0.5	1.5			0.5	

Polar axis

Experiment N°	20.0	22.5	25.0	27.5	30.0	32.5	35.0	37.5	40.0	42.5	45.0	47.5	50.0	µm
T-12-289	0.5		12.5	44.0	33.5	7.5	1.5	0.5						%
T-12-290		1.0	12.5	38.0	36.0	12.0	0.5							
T-12-291			5.5	46.5	38.0	6.5	3.5							
T-12-292		1.5	11.5	26.0	34.0	20.5	5.0	1.0	0.5					
T-12-293		0.5	7.5	24.0	36.5	22.5	8.0	1.0						
T-12-306A			2.0	8.0	38.0	36.0	12.0	3.0	0.5	0.5				
T-12-306B			2.5	3.5	16.5	27.5	33.5	12.0	2.5			1.0	1.0	
T-12-307A		0.5	2.5	10.5	28.5	36.5	17.0	4.0			0.5			
T-12-307B		0.5	1.5	10.0	24.5	38.0	19.0	4.0	1.0	0.5	1.0			
T-12-308A				2.0	25.5	33.0	30.5	8.0	0.5	0.5				
T-12-308B				8.0	44.0	42.5	4.5	1.0						
T-12-309A			2.0	13.5	39.0	37.5	7.0	1.0						
T-12-309B				3.0	37.5	44.5	12.5	2.5						
T-12-310A				2.5	12.0	42.0	31.5	9.5	2.5					
T-12-310B				0.5	9.0	37.5	43.0	7.5	2.5					
T-12-311A				1.0	14.5	40.5	33.5	9.0	1.5					
T-12-311B				2.0	17.0	35.5	35.5	7.5	2.5					

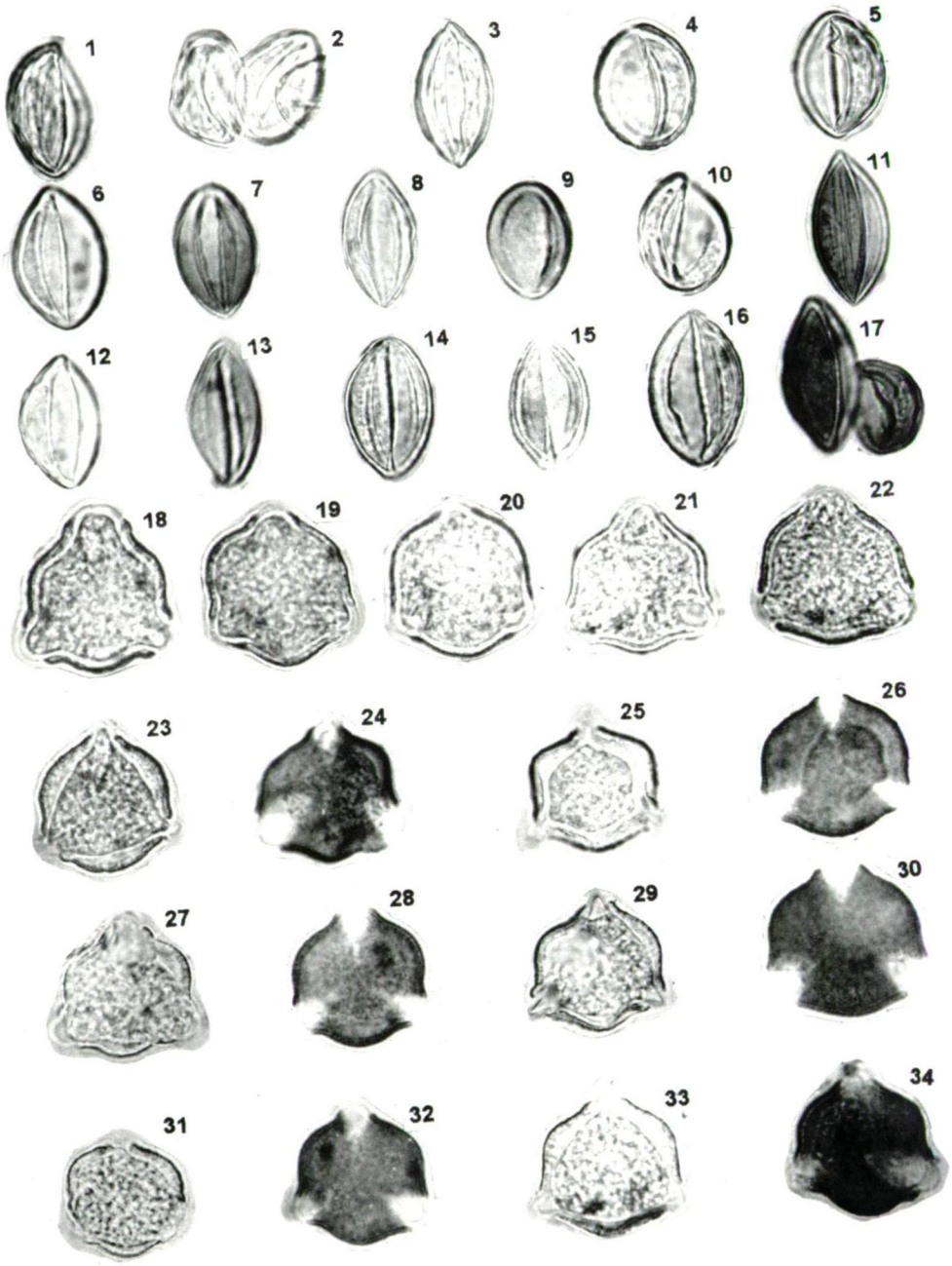
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Plate 7.1.

Plate 7.1.

1-17. *Gingko biloba* L. 1. fresh pollen grain, 2--5..Pollen grains heated on 30 °C. 2. length of time:30 minutes, 3. length of time: 1 hour, 4. length of time: 5 hours, 5. length of time:10 hours. 6-17. Pollen grains partially degraded with C60 fullerene/benzol solution. 6,7. length of time: 1 day, 6. unstained, 7. stained pollen grain, 8,9. length of time: 2 days, 8. unstained, 9. stained pollen grains

18-34. *Quercus robur* L. 18. fresh pollen grain. 19-22. pollen grains heated on 30 °C.19. length of time: 30 minutes, 20. length of time: 1 hour, 21. length of time: 5 hours, 22. length of time:10 hours. 23-34. Pollen grains partially degraded with C60 fullerene/benzol solution. 23,24. length of time: 1 day, 23 unstained, 24. stained pollen grain. 25,26. length of time:2 days. 25. unstained, 26. stained pollen grain. 27,28. length of time: 3 days, 27. unstained, 28. stained pollen grain. 29,30 length of time: 4 days. 29. unstained, 30. stained pollen grain. 31,32. length of time: 5 days.31. unstained, 32. stained pollen grain. 33,34. length of time: 6 days, 33. unstained, 34. stained pollen grain.

The characteristic LM morphology of these pollen grains has not changed after heating (Plate 7.1., figs. 2-5) and after the treatment with C60 fullerene/benzol solution. But there are some alterations in the P/E ratio (Plate 7.1., figs. 6-17). 1.3 and 1.4 was the maximum value at the fresh dry pollen grains, after heating for 10 minutes 1.2, 1.3 was measured. These values were more or less constant at the further experiments.

Quercus robur L. (Plate 7.1., figs. 18- 34)

Pollen gains of this species were observed and investigated in polar position. The diameter was measured.

Diameter

Experiment N°	20.0	22.5	25.0	27.5	30.0	32.5	35.0	37.5	µm
T-12-284			2.0	2.9	53.0	13.0	3.0		%
T-12-285				18.5	65.0	16.5			
T-12-286			1.5	16.5	44.5	33.5	4.0		
T-12-287			1.0	30.0	42.0	14.0	11.5	1.5	
T-12-288			2.0	33.5	56.0	8.5			

The maximal values of the diameter decreased after 5 and 10 hours of heating.

T-12-300A		3.5	45.5	36.0	15.0			
T-12-300B	0.5	6.0	15.0	29.5	38.5	9.0	1.5	
T-12-301A		4.0	14.5	25.0	35.0	17.0	4.5	
T-12-301B			5.5	17.0	41.5	29.0	7.0	
T-12-302A		2.0	11.0	30.5	34.0	17.0	5.5	
T-12-302B		1.0	11.0	15.5	37.0	30.0	5.0	0.5
T-12-303A		3.0	12.0	33.0	43.5	8.0	0.5	
T-12-303B		0.5	8.5	19.0	35.5	29.0	7.5	
T-12-304A			2.0	24.5	45.5	19.0	9.0	
T-12-304B			10.5	22.5	39.0	21.0	6.5	0.5
T-12-305A			7.0	22.5	43.0	22.0	5.5	
T-12-305B			2.0	24.5	43.0	26.0	4.5	

The maximum values of the diameter of the pollen grains after treatment with C60 fullerene/benzol solution increased Sometimes there are differences between the unstained and stained pollen grains at the same experiment. After 5 and 6 days the maximum, values are essentially identical.

Plate 7.2.

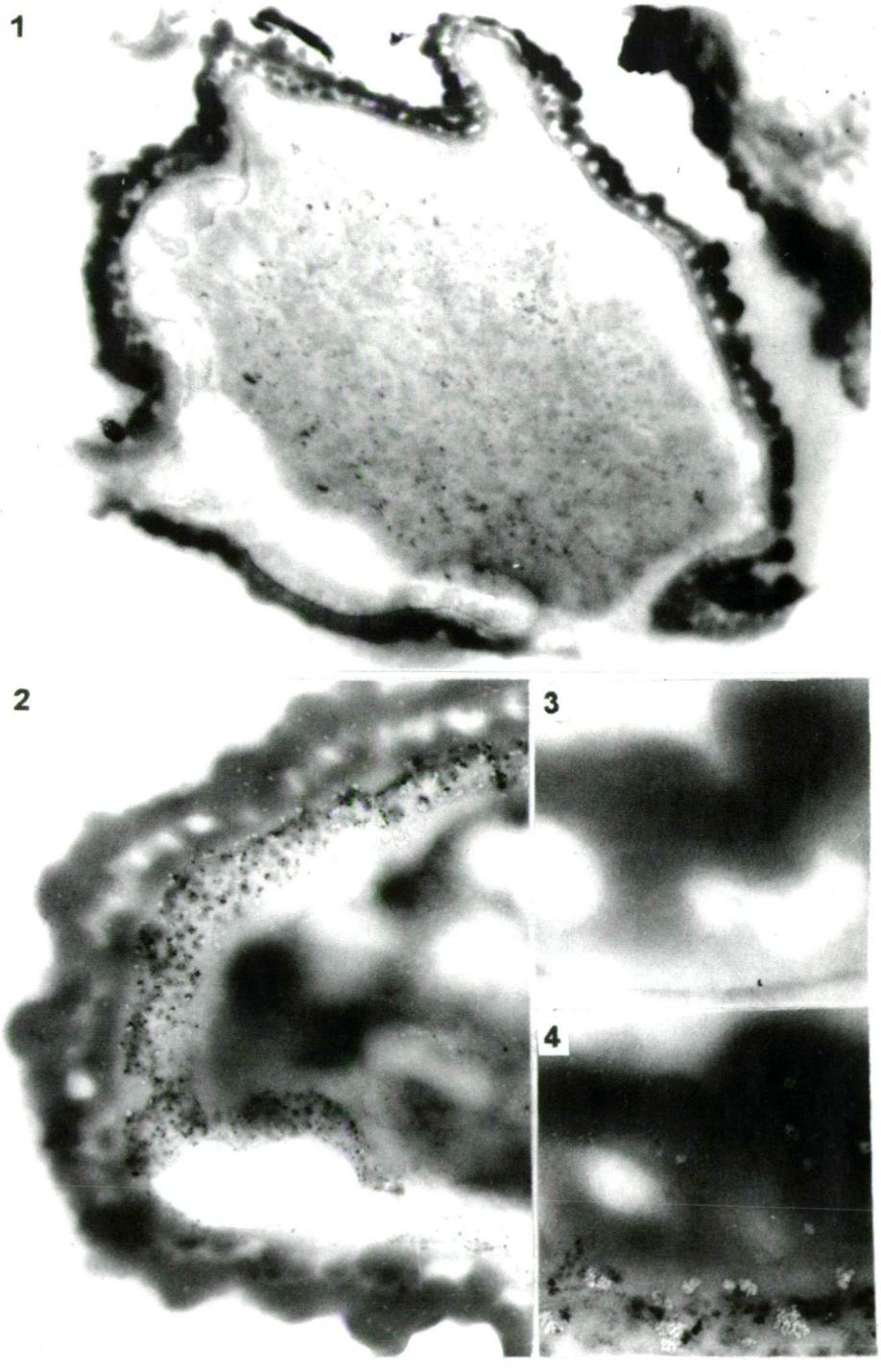

Plate 7.3.

Plate 7.2.

1-7. *Ginkgo biloba* L. Transmission electron microscopy of partially degraded pollen grains with C60 fullerene/benzol solution during 1 day.
1,2,5. General survey picture from the equatorial plane sectioned pollen grains 5.000x., 1. Negative No.: 9621, 2. negative No.: 9592, 5. Negative No.: 9624.
3,4. Details from the exine ultrastructure. 15.000x. 3. negative No.: 9622, 4. Negative No.: 9593.
6,7. Details from the partially degraded ectexine. 50.000x. 6. Negative No.: 9556, 7. Negative No.: 9589.

Plate 7.3.

1-4. *Quercus robur* L. Transmission electron microscopy of partially degraded pollen grains with C60 fullerene/benzol solution during 1 day.
1. General survey picture of the ultrastructure of the pollen grain in equatorial section. 5.000x, Negative No.: 9626.
2. Detail from the exine and protoplasm of the partially degraded pollen grain. 15.000x. Negative No.: 3617.
2,4. Detail from the ultrastructure of the pollen wall. 50.000x. 3. Negative No.: 9669. 4. Negative No.: 9819.

TEM results

Ginkgo biloba L. (Plate 7.2., figs. 1-7)

The ultrastructure of the pollen grains was investigated in equatorial sections. The general survey pictures (Plate 7.2., figs. 1, 2, 5) illustrate well the electron dense ectexine and the protoplasm. The thin apertural ectexine (sulcus wall) is well shown in figs. 2 and 4, (Plate 7.2). The ectexine stratification is more or less well preserved, the tectum, infratectal layer and the foot layer are perceptible. Dissolution or degradation of biopolymer units of the ectexine was not observed (Plate 7.2., figs. 6,7).

Quercus robur L. (Plate 7.3., figs. 1-4)

The general survey picture (Plate 7.3., fig. 1) illustrate well the dark electron dense ectexine, the light intine and the more or less degraded granular protoplasm. The ultrastructure of the ornamental elements of the tectum, the columellar infratectal layer and sometimes the dark ectintine are well shown. Fine structure of the ectexine is illustrated in picture 3 and 4 (Plate 7.3). Well shown are in picture 4, and 2, the light holes at the border of the foot layer and ectintine, and in the intine, indicates the dissolution of globular biopolymer units from this part of the wall. The dark globular units in the intine may be the consequence of the partial dissolution or degradation of the molecular system of this layer.

Discussion and Conclusions

The pollen subjected to a 30 °C temperature did not have a meaningful effect on these grains. There was only a moderate change in the P/E ratio of *Ginkgo biloba* pollen. Therefore, it can be assumed that during wind dispersion the air temperature is not an important factor in changing the morphological characters of these pollen grains.

The TEM data of the partially dissolved pollen grains with the C60 fullerene/benzol solution suggest that the technique is useful and promising for future studies. This technique reveals the ultrastructure elements without the usual fixation of glutar-aldehyde and the postfixation with OsO_4 aq. dil. This technique was succesful in both the relatively resistant ectexinous pollen of *Ginkgo biloba* and the less resistant *Quercus robur.*

As first step in this type of experiment, the results of both the homogeneous consistence of the ectexine and the electron dense protoplasm of *Ginkgo biloba* are also consistent with our early observations on *Taxus baccata* L. pollen.

It is out belief that the partial dissolution of the biopolymer structures at the outermost part of the foot layer and the innermost part of the ectintine indicate the complexity of the biopolymer system of the exinous pollen wall. The tectum, infratectal layer and the outer part of the foot layer are resistant to this treatment. In another experiment (KEDVES, PÁRDUTZ and VARGA, 2002) we observed a separate electron dense part of the foot layer and perhaps also the endexine. The origin of this latter mentioned layer needs further investigation and it may be identified with a less resistant molecular system.

Acknowledgements

The writers are grateful to Professor Edward A. STANLEY (Ardmore, PA, U.S.A.) for critically reading the manuscript and for his valuable comments. This work was supported by Grant OTKA T 31715.

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8. EXPERIMENTAL INVESTIGATIONS ON THE POLLEN GRAINS OF MALVA SYLVESTRIS L. AND HIBISCUS SYRIACUS L. I.

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Abstract

The allergenic pollen grains of *Malva sylvestris* L. and as comparative material, that of *Hibiscus syriacus* L., were partially degraded with 2-aminoethanol during 30 minutes, 1, 5, 10 and 24 hours and investigated by the LM method. Dissolution of the mucilage drops and alterations in the general aspect of the pollen morphology, diameter, length of spinae and the size of the apertures were investigated. In general pollen grains of both species are resistant to the experimental processes employed. Important alterations in the general aspect of the pollen grains were observed after 10 hours and in particular, 24 hours of treatment.

Key words: Experimental Palynology, recent, Malvaceae, LM method.

Introduction

The characteristic pollen grains of the family, Malvaceae have been the subject of several studies: following THANIKAIMONI (1972, 1973) the first palynological data from the genus *Malva* were published by FRITZSCHE (1832) and MOHL (1835). For the genus *Hibiscus* the bibliographical data of FRITZSCHE (1832) and HASSALL (1842) may be mentioned based on the Bibliographical index of THANIKAIMONI (1972, 1973). Several bibliographical data and LM morphology of the Malvaceae pollen grains were published by ERDTMAN (1952) also. As important papers in this subject the following are selected: LANG (1937), ERDTMAN and VISHNU-MITTRE (1958), ERDTMAN (1959), SAAD (1960), ERDTMAN, BERGLUND and PRAGLOWSKI (1961), FREYTAG (1964), GULLVÄG (1964), SOWUNMI (1973), UENO (1978), GOETZ (1982), CHRISTENSEN (1986A), CULHANE and BLACKMORE (1988) and LA SERNA RAMOS and DOMINGUEZ SANTANA (1991). In the monograph of CHESTER and RAINE (2001) there are important informations on the Malvaceae pollen grains too.

This object of the investigations is contribution to the ongoing studies of pollen degradation in various taxa.

Materials and Methods

The investigated material was collected in Szeged on the 22.09.2000, by Miss K. PRISKIN, and Miss Zs. IMRE 30 stamens were used for the experiments. Experimental parameters inwolved were: Temperature (30 °C), length of time (30 minutes, 1, 5, 10 and 24 hours). The pollen grains after washing were mounted in glycerine jelly hydrated for 39.6% (LOBREAU, 1966). The following characters were measured: diameter of the pollen grains, length of the spinae and the size of the apertures.

General problems

Following KHAN (1992) the Malvaceae originated in the southern Gondwana continent and underwed speciation at two centres, Neotropical and Australian-Oceania. The evolutionary trend of the Malvaceae pollen grains is from ancestral tricolporate pollen type with small spines to a large polytreme type with long spines (CHRISTENSEN 1986b). NAIR (1958) established, that the pollen grains of *Malva parviflora* LINN. collected from Kashmir are ornamented with two types of spines (those with pointed apices, and those with blunt apices). He pointed out, that it is difficult to explain the dimorphism, but as a possible explanation is that, this is a consequence of some meiotic disturbance. SRIVASTAVA (1982) compiled an evolutionary trend of spines of the different taxa of the Malvaceae, as follows: blunt tips to pointed tips. Unbranched to branched. HANKS and DRYXELL (1979) investigated 9 characters of some Malvaceae pollen grains. MATEU, GÜEMES and SALVADOR (1988) used the following characters during its investigations on the pollen grains of Malvaceae from Valencia: diameter, characters of the spinae diameter of the apertures. Interesting schemas of the spinae morphology were published. LA SERNA RAMOS and DOMINGUEZ SANTANA p. 106 (1991) who used the pollen biometry method of different populations of the species of the genus *Lavathera* L. in the Canary Islands. New morphological data from the pollen grains of *Hibiscus syriacus* L. were published by MAR TRIGO, GARCIA and CABEZUDO (1994).

TAKAHASHI and KOICHI (1988) investigated with the LM, SEM and TEM methods the ontogenetic development of the spinous exine of *H. syriacus* with special reference to the formation of the spines. The mature pollen grain is polycolporate, 160-170 μm in diameter, with supratectal spines 10-25 μm long.

KNOX and HESLOP-HARRISON (1969) investigated the cytochemical localization of enzymes in the wall of the pollen grains of several species, among which were malvaceous species, *Malvaviscus arboreus* and *Hibiscus rosa-sinensis*. DIAZ DE LA GUARDIA et al. (1994) described osmiophyl granules on the surface and the holes of the infratectal layer of the pollen grain of *Lavatera oblongifolia* BOISS.

We believe that the above mentioned and incomplete review concerning this subject, support the importance of the investigations of the pollen grains of the Malvaceae. In this place we have not discussed the experimental and the electronmicroscopic results, because such investigations are in progress.

Results

1. *Malva sylvestris* L.

1. Fresh pollen grains (Plate 8.1., figs.1-3)

Amb circular, polyaperturate tectum ornamented with spines. Mucilage drops are well shown. Diameter of the investigated pollen grains: 75.0-100.0 μm, maximum: 82.5-87.5 μm. Length of the spinae: 5.0 - 10.0 μm, maximum: 7.5 μm. Aperture size: 5.0-10.0 μm, maximum: 7.5 μm.

Pollen grains partially degraded with 2-aminoethanol

2.1. Degradation for 30 minutes (Plate 8.1., figs. 4-6)

No important alterations in the general morphology of the pollen grains except the partial dissolution of the mucilage drops. Diameter: 80.0-100.0 μm, maximum: 85.0-92.5 μm. Length of the spinae: 6.25-10.0 μm, maximum: 7.5 μm. Aperture size: 5.0-10.0 μm, maximum: 7.5 μm.

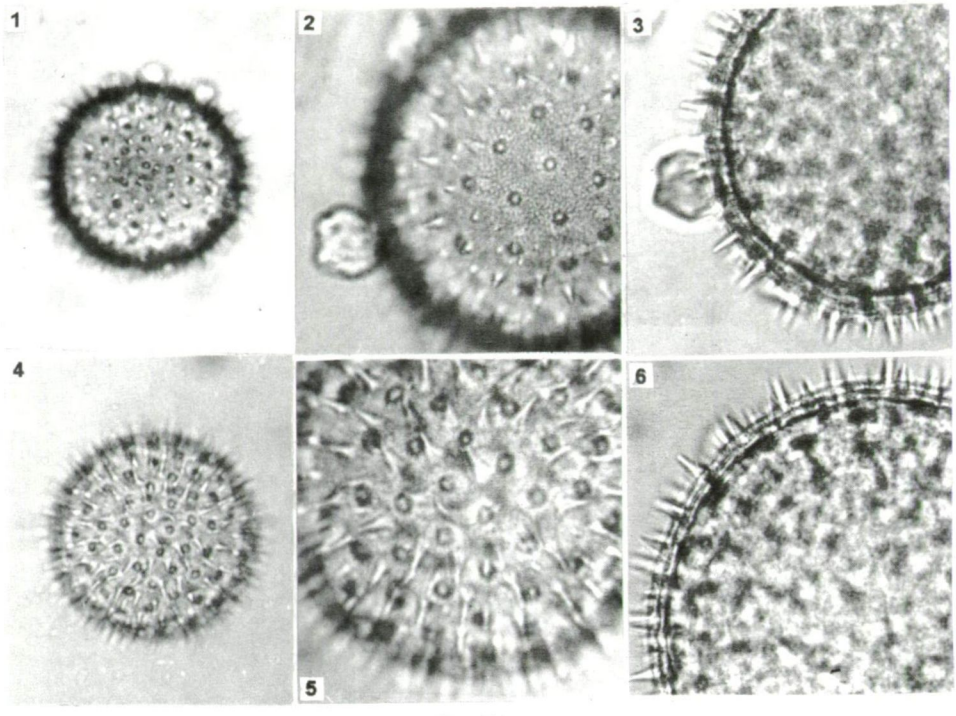

Plate 8.1.

1-6. *Malva sylvestris* L.
1-3. Fresh pollen grains
4-6. Pollen grains partially degraded with 2-aminoethanol for 30 minutes. Magnifications: 1, 4. 330x., 2,3,5,6. 660x.

2.2. Degradation for 1 hour (Plate 8.2., figs. 1-3)

The general aspect of the pollen grains is identical to the fresh ones, but the mucilage drops dissolved. Diameter: 77.5 - 105.0 µm, maximum: 85.0 - 95.0 µm. Length of spinae: 5.0 - 12.5 µm, maximum: 7.5 µm. Aperture size: 5.0 - 12.5 µm, maximum: 7.5 µm.

2.3. Degradation for 5 hours (Plate 8.2., figs. 4-6)

The qualitative morphological characteristic features are identical to the previous experiment. Diameter: 80.0 - 125.0 µm, maximum: 97.5 - 110.0 µm. Length of spinae: 5.0 - 11.5 µm, maximum: 7.5 µm. Aperture size: 5.0 - 10.0 µm, maximum: 7.5 µm.

Plate 8.2.

1-12. *Malva sylvestris* L.
1-3. Pollen grains partially degraded with 2-aminoethanol for 1 hour.
4-6. Pollen grains partially degraded with 2-aminoethanol for 5 hours.
7-9. Pollen grains partially degraded with 2-aminoethanol for 10 hours.
10-12. Pollen grains partially degraded with 2-aminoethanol for 24 hours. Magnifications: 1, 4, 7, 10. 330x, 2, 3, 5, 6, 8, 9, 11, 12. 660x.

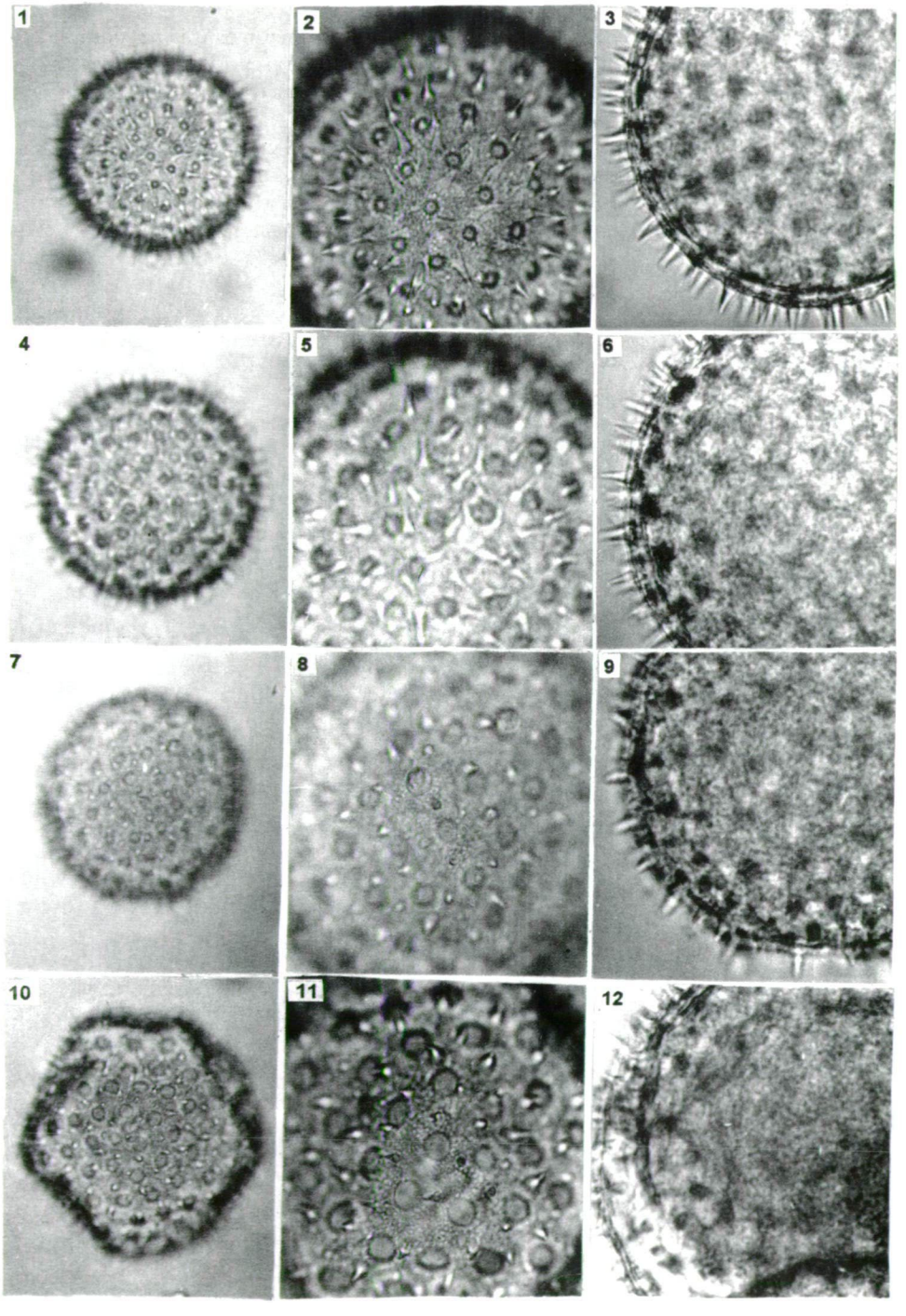

Plate 8.2.

2.4. Degradation for 10 hours (Plate 8.2., figs. 7-9)

The globular form of the pollen grains altered slightly, sometimes it is not completely globular. Diameter: 77.5 - 130.0 µm, maximum: 85.0 - 107.5 µm. Length of spinae: 5.0 - 8.75 µm, maximum: 7.5 µm. Aperture size: 5.0 - 8.75 µm, maximum: 6.25 µm.

2.5. Degradation for 24 hours (Plate 8.2., figs. 10-12)

Remarkable deformation of the originally globular pollen grain was observed, illustrated in picture 10, of Plate 8.2. Diameter: 90.0 - 130.0 µm, maximum: 112.0 - 122.5 µm. Length of spinae: 5.0 - 10.0 µm, maximum: 7.5 µm. Aperture size: 5.0 - 10.0 µm, maximum: 7.5 µm, but the number of the apertures of 10.0 µm large are also frequent.

2. *Hibiscus syriacus* L.

1. Fresh pollen grains (Plate 8.3., figs. 1-3)

Polyaperturate, globular pollen grains ornamented with large spines. Numerous characteristic mucilage drops were observed. Diameter: 105.0 - 170.0 µm, maximum: 132.5 - 142.5 µm. Length of spinae: 12.5 - 27.5 µm, maximum: 15.0 - 17.5 µm. Aperture size: 14.0 - 22.5 µm, maximum: 12.5 - 15.0 µm.

2. Partially degraded pollen grains with 2-aminoethanol

2.1. Degradation for 30 minutes (Plate 8.3., figs. 4-6)

The general aspect of the pollen grain is similar to the fresh ones, except the mucilage drops were partially dissolved. Diameter: 97.5 - 170.0 µm, maximum: 117.5 - 125.0 µm. Length of spinae: 10.0 - 25.0 µm maximum: 13.0 - 17.5 µm. Aperture size: 7.5 - 22.5 µm, maximum: 12.5 - 15.0 µm.

2.2. Degradation for 1 hour (Plate 8.3., figs. 7-9)

Amb circular, alterations were not observed by the LM method. It is worth of mentioning that the mucilage drops are still present and more common than at the previous experiment. Diameter: 110.0 - 147.5 µm, maximum: 125.0 - 132.5 µm. Length of spinae: 12.5 - 22.5 µm, maximum: 15.0 µm. Aperture size: 10.0 - 20.0 µm, maximum: 12.5 - 15.0 µm.

2.3. Degradation for 5 hours (Plate 8.4., figs. 1-3)

The general aspect of the pollen grains have not altered during this experiment, but the mucilage drops are mostly dissolved. Diameter: 117.5 - 157.5 µm, maximum: 125.0 - 142.5 µm. Length of spinae: 12.5 - 25.0 µm, maximum: 17.5 µm. Aperture size: 10.0 - 22.5 µm, maximum: 12.5 - 15.0 µm.

2.4. Degradation for 10 hours (Plate 8.4., figs. 4-6)

Deformations started at this experiment. The ectexine wrinkled, the amb is not always circular, sometimes slightly angular. Mucilage drops dissolved completely. Diameter: 100.0 - 150.0 µm, maximum: 125.0 - 132.5 µm. Length of spinae: 10.0 - 25.0 µm, maximum: 15.0 - 17.5 µm. Aperture size: 7.5 - 22.5 µm, maximum: 10.0 - 15.0 µm.

2.5. Degradation for 24 hours (Plate 8.4., figs. 7-9)

Important deformations were observed after this experiment. The amb is polyangular. On the surface protrusions appeared sometimes similar to the dissolved mucilage drops. Diameter: 107.5 - 152.5 µm, maximum: 120.0 - 132.5 µm. Length of spinae: 12.5 - 22.5 µm, maximum: 15.0 - 17.5 µm. Aperture size: 7.5 - 20.0 µm, maximum: 10.0 µm.

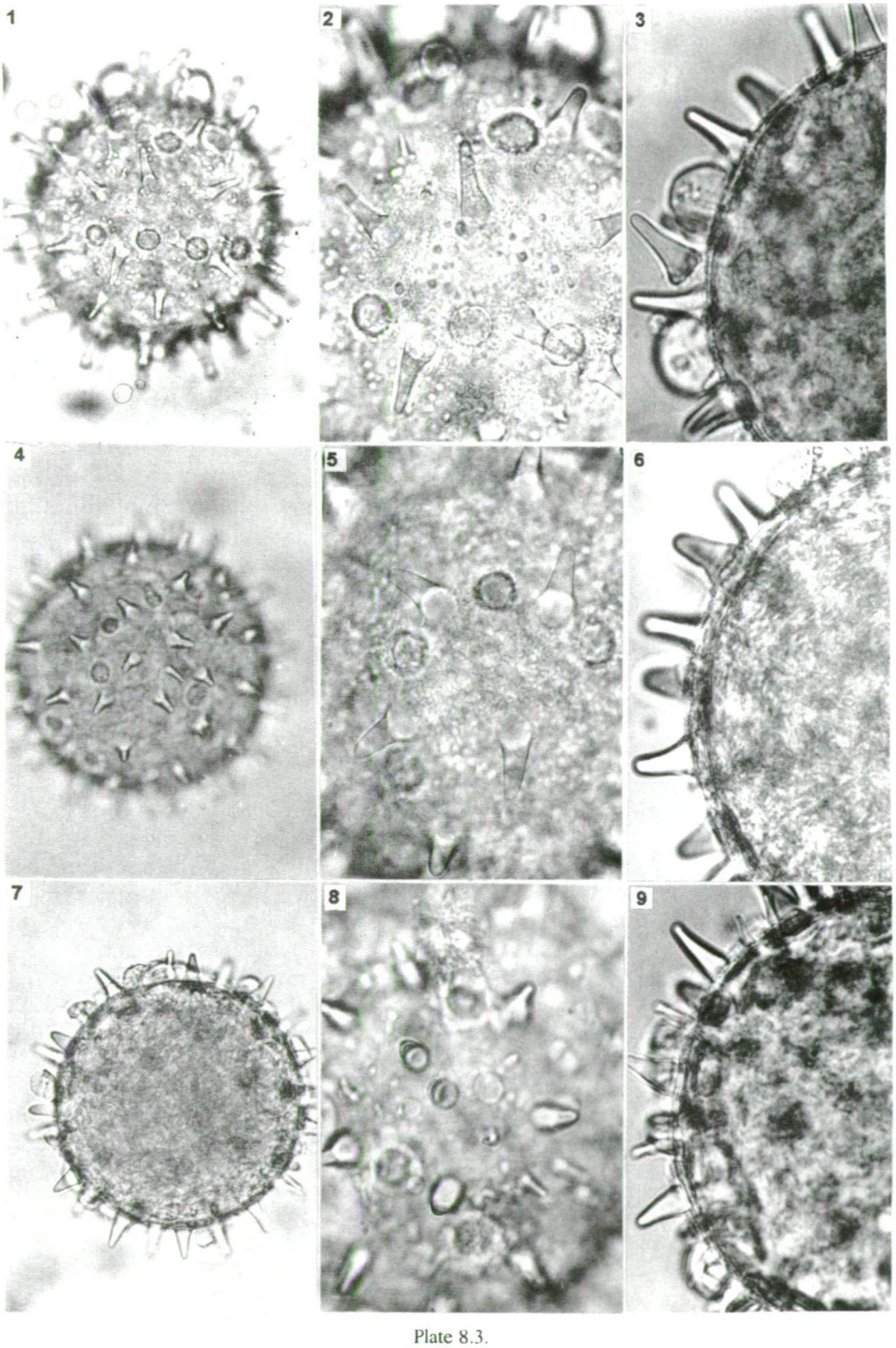

Plate 8.3.

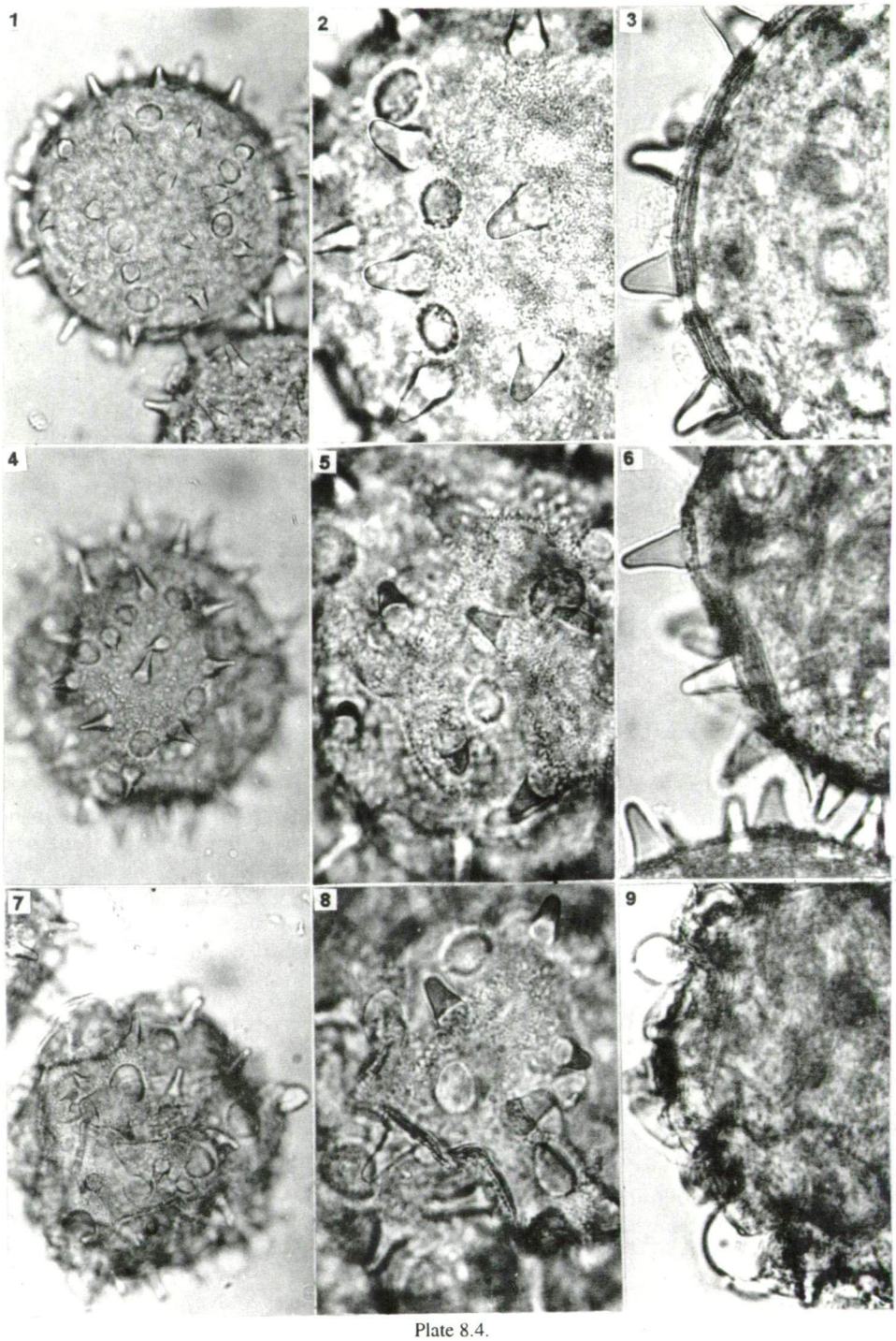

Plate 8.4.

Plate 8.3.

1-9. *Hibiscus syriacus* L.
1-3. Fresh pollen grains.
4-6. Pollen grains partially degraded with 2-aminoethanol for 30 minutes.
7-9. Pollen grains partially degraded with 2-aminoethanol for 1 hour. Magnifications: 1, 4, 7, 330x, 2, 3, 5, 6, 8, 9. 660x.

Plate 8.4.

1-9. *Hibiscus syriacus* L.
1-3. Pollen grains partially degraded with 2-aminoethanol for 5 hours.
4-6. Pollen grains partially degraded with 2-aminoethanol for 10 hours.
7-9. Pollen grains partially degraded with 2-aminoethanol for 24 hours. Magnifications: 1, 4, 7, 330x, 2, 3, 5, 6, 8, 9. 660x.

Discussion and Conclusions

1. The sporopollenin of the ectexine of both species investigated are relatively resistant to other species investigated previously for example some species of the genus *Quercus*, and *Elaeagnus angustifolia*. Relatively long degradation is necessary to observe important morphological alterations at these pollen grains.

2. Regarding the dissolution of the characteristic mucilage drops of these pollen grains based on our present day data the mucilages of *H. syriacus* are more resistant than that of *M. sylvestris*. The importance of these drops in the allergenic character of the pollen grains is in question.

3. Regarding the quantitative data of *M. sylvestris* there are no important differences in the measured characteristic features. Probably the maximum of the diameter is a bit larger after partial degradation for 24 hours. Worth of mentioning is that the maximum of the length of the spinae is 7.7 μm. Small differences are at the size of the apertures. 10 μm large aperture was observed after 24 hours of degradation.

4. At the pollen grains of *H. syriacus* some differences were established in the quantitative data of the partially degraded pollen grains. Concerning the diameter of the pollen grains it is worth of mentioning, that TAKAHASHI and KOICHI (1988) published 160-170 μm, and for the fresh pollen grains from Hungary (Szeged) our data are: 105.0 - 170.0 μm.

5. SEM investigations of these partially degraded pollen grains and the TEM study of partially degraded pollen grains of *M. sylvestris* by C60 fullerene/benzol solution will be published before long.

6. Later, in possession of the EM data we will have the opportunity to establish without doubt the character of the apertures, which may be polyporate, polyforate or (brevi)polycolporate with short colpi. After the establishment of the polycolporate condition by TAKAHASHI and KOICHI (1988) for the pollen grains of *H. syriacus* this will be investigated again.

7. Finally we will take into consideration the findings of ERDTMAN, BERGLIUND and PRAGLOWSKI (1961): Pollen dimorphism seems to occur at least in certain specimens of *M. pusilla* (some grains have long pointed spines, other grains shorter with blunt processes (p. 40).

Acknowledgements

The authors are grateful to Eric CAULTON Director, Scottish Centre for Pollen Studies, Edinburgh, U.K., for his comments and linguistic corrections of the text. This work was supported by Grant OTKA T 031715.

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9. LM AND TEM INVESTIGATIONS ON PARTIALLY DEGRADED POLLEN GRAINS OF TRITICUM AESTIVUM L.

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Abstract

Pollen grains of *Triticum aestivum* L. were partially degraded with four series of experiments: 1. Degradation with 2-aminoethanol for 24, 48 and 72 hours. 2. After the treatment mentioned previously, $KMnO_4$ (1%) was added for 24 hours. 3. In this experiment merkaptoethanol was added to the residous after degradation with 2-aminoethanol. 4.Pollen grains partially dissolved with glycerine aq. dil. (50%). LM and TEM methods were used.

Key words: Experimental Palynology, *Triticum aestivum*, LM, TEM.

Introduction

Triticum is an extremely important genus from several points of view. According to the "Index bibliographique" of THANIKAIMONI (1972, 1973) pollen grains of this genus were first investigated by MOHL (1835) and EDGEWORTH (1877). Important findings were published by ERDTMAN (1944, 1956), using the LM method. Phase contrast microscopy was applied by several researchers e.g. ERDTMAN and PRAGLOWSKI (1959), ERDTMAN, BERGLUND and PRAGLOWSKI (1961). FAEGRI and IVERSEN (1964) emphasized the importance of the phase contrast characteristics of the pollen grains in the differentiation of various cereals and wild grasses. SORSA (1968) used the interference contrast method for the pollen grains of *Triticum aestivum*. The organization and the polarity of the pollen mother cells were studied by DOVER (1972).

TEM studies. - The ultrastructural characteristic features of the pollen development of *Triticum aestivum* was investigated by SHIH-YI, MO-SHAN and LI-YUN (1977). The correlations between the development of the aperture and the interapertural part of the pollen grains were investigated by EL-GHAZALY and JENSEN (1985, 1986a,b). Comparison of exine development in normal and gametocide treated plants were investigated with the TEM method by EL-GHAZALY (1990). Cytological and genetical aspects in anther culture were investigated by SZAKÁCS (1992).

ANDERSEN and BERTELSEN (1972) used the scanning electron microscope for the pollen grains of cereals and other grasses. In this paper the importance of the identification of the fossil pollen of cereals in studies of postglacial vegetational history was emphasized. KÖHLER and LANGE (1979) published further data in this respect. The SEM method was used for selected Triticinae and intergeneric hybrids by RAJENDRA, TOMB,

MUJEEB and BATES (1978), and the usefulness of micromorphological pollen characteristics as genetic markers was established.

Taking into consideration the allergenic characters of the grass pollen grains, including the cereals (e.g.: RICHARD et al., 1986, DE LEONARDIS et al., 1986, NILSSON, PRAGLOWSKI and NILSSON, 1977, LEBBE et al., 1988, etc.), we also included the pollen grains of *Triticum aestivum* also within the experimental research program of our laboratory on allergenic pollen grains.

The aim of this paper is to establish the pollen morphological and ultrastructural alterations as a consequence of the experimental influences, in comparison to the previous results in this subject.

Materials and Methods

The investigation material (*Triticum aestivum* L. cv. GK-Kalász) was collected by Dr. A. PALÁGYI and Mrs. B. VARGA on the 18th May 2001 Locality: Ságvári Experimental Research Station of the Cereal Research Institute, Szeged.

Fresh grains, (T-12-230) unstained (A) and stained with Methylviolet (B) were investigated. The experiments were as follows:

1. Treatment with 2-aminoethanol during 24, 48 and 72 hours, experiment numbers T-12-231, 232, 233.
2. Treatment with 2-aminoethanol as previously, but after this $KMnO_4$ (1%) was added for 24 hours.
3. Treatment with 2-aminoethanol as previously (1), after this 2 ml merkaptoethanol was added for the exinous remnants for 24 hours.
4. Partial dissolution with glycerine aq. dil. (50%) for 30 days. The pollen grains were mounted in glycerine-jelly, hydrated at 39.6%, and/or in Araldite. Embedding for TEM studies in Araldite after postfixation with OsO_4 aq. dil.(1%) The ultrathin sections were made with glass knives on a Porter Blum ultramicrotome in the Cell Biological and Evolutionary Micropaleontological Laboratory. The pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences on a Tesla BS 540 instrument. All pictures are unretouched..

Results

Percentages of the diameter of the pollen grains investigated.

µm	45.0	47.5	50.0	52.5	55.0	57.5	60.0	62.5	65.0	67.5	70.0	72.5	75.0	77.5
T-12-230A						2.5	18.0	28.5	22.0	16.0	10.0	3.0		
T-12-230B			12.0	27.5	25.5	20.0	12.5	2.5						
T-12-231A			1.0	4.0	10.5	28.5	22.0	22.0	10.0	1.5		0.5		
T-12-231B		0.5	3.0	8.5	13.0	31.0	24.5	16.5	3.0					
T-12-231Ar					2.5	12.0	22.0	33.5	21.5	6.5	2.0			
T-12-232A			1.0	1.5	7.0	13.0	17.0	32.5	15.0	11.5	1.5			
T-12-232B			0.5	2.0	6.5	15.0	26.0	25.0	12.5	9.0	3.5			
T-12-232Ar					3.0	6.5	18.0	24.0	23.0	15.5	6.5	3.5		
T-12-233A		1.0	3.0	3.5	7.5	21.0	24.5	26.5	10.0	2.5		0.5		
T-12-233B		1.5	12.5	19.0	21.5	21.5	13.5	10.0	0.5					
T-12-233Ar				2.5	10.5	19.5	21.5	27.0	12.5	6.5				
T-12-234A			4.5	4.5	15.5	20.0	13.0	19.0	6.0	9.0	5.0	1.5	2.0	
T-12-234Ar							10,0	14.0	15.5	23.0	18.5	11.0	6.0	2.0
T-12-235A		1.0	6.5	13.0	29.0	27.0	12.5	9.5	1.0	0.5				
T-12-235Ar					4.0	13.5	21.5	19.0	21.0	14.0	5.0	1.5	0.5	
T-12-236A		2.5	13.5	20.0	23.5	20.5	9.0	4.5	0.5	3.0	0.5	2.0	0.5	
T-12-236Ar						6.0	25.0	25.0	20.0	10.0	11.5	2.5		
T-12-237A		1.5	3.5	7.0	21.5	23.0	23.0	15.0	5.0	0.5				
T-12-237B					7.5	14.5	23.5	27.0	14.0	7.0	2.5	3.0	1.0	

µm	45.0	47.5	50.0	52.5	55.0	57.5	60.0	62.5	65.0	67.5	70.0	72.5	75.0	77.5
T-12-237Ar	0.5		1.0	6.0	14.5	24.0	30.0	13.5	8.0	2.5				
T-12-238A		2.0	4.5	16.0	14.0	28.5	18.5	12.5	4.0					
T-12-238B				2.5	15.5	26.5	28.0	25.5	2.0					
T-12-238Ar					12.0	12.5	23.5	27.0	15.5	9.5				
T-12-239A			0.5	3.0	7.5	19.0	27.0	35.5	7.5					
T-12-239B		0.5		0.5	2.5	4.5	10.5	16.0	16.0	21.0	16.5	7.5	4.5	
T-12-239Ar				0.5	3.0	9.5	19.0	32.0	20.5	12.5	3.0			
T-12-240A			5.0	11.5	19.5	25.0	24.0	14.5	0.5					
T-12-240B	0.5	0.5	2.0	11.0	26.0	29.5	23.0	7.0	0.5					
T-12-240Ar	1.5	6.0	20.0	33.5	22.0	12.0	3.0	1.5	0.5					

Table 9.1.

1. Fresh, untreated pollen grains (Plate 9.1., figs. 1-4, table 9.1)

Typically monoporate pollen grains. Diameter of the non-stained pollen grains from 57.5-72.5 µm, maximum (28.5%) at 62.5 µm. The stain changed the size of the pollen grains: diameter from 50,0 - 62.5 µm, maximum (27.5%) at 52.5 µm.

2. Partially degraded pollen grains with 2-aminoethanol (Plate 9.1., figs. 5-15, plate 9.2., figs. 1-6)

2.1. Experiment No.: T-12-231, length of time: 24 hours (Plate 9.1., figs. 5-10)

LM results (Plate 9.1., figs. 5-8, table 9.1.). The basic morphology of the pollen grains has not changed, only the protrusions are characteristic. The variation in the diameter of the non-stained and the stained pollen grains is nearly identical, but the embedding effect increased the size of the pollen grains.

TEM results.(Plate 9.1., figs. 9,10) The inter-apertural exine (Plate 9.1., fig. 9) is degraded. No orbiculi were observed on the surface of the tectum. Channels of the tectum are not so characteristic. Secondary thinning of the elements of the infratectal layer are perceptible. Well shown is the destruction of the outer part of the intine. In the apertural area (Plate 9.1., fig. 10), the elements of the annular system are more or less homogenized. Radially oriented light holes were present, which may indicated the presence of helical biopolymer systems.

2.2. Experiment No.: T-12-232, length of time 48 hours. (Plate 9.1., figs. 11-15, Plate 9.2., fig.1)

LM results (Plate 9.1., figs. 11-13, table 9.1.) No qualitative deformations were observed in the partially degraded pollen grains. In consequence of the stain, the diameter diminishes, but the size of the embedded pollen grains is nearly identical with the non-stained ones.

TEM results (Plate 9.1., figs. 14,15, plate 9.2., fig. 1) The degradation of the infratectal layer is characteristic in the inter-apertural ectexine (Plate 9.1., fig. 15). In the general survey picture (Plate 9.1, fig. 14) of the exine, remnants of the intine are illustrated. This layer was secondarily thickened and a degraded electron dense layer is located within the more or less homogeneous inner layer. The fine structure in the apertural area is more resistant than in the inter-apertural area (Plate 9.2., fig.1). The ectexinous elements of the operculi are well shown, as electron dense particles on a thin lamellar layer. The endannulus is homogeneous, the thickened ectintine is granular and a little less electron dense than the foot layer. Near the apertural area, the ectintine is well preserved and the inner border is wavy. The endintine is probably degraded. The ultrastructure of the protrusion is lamellar or irregular and, the elements are more or less oriented in the direction of the pore.

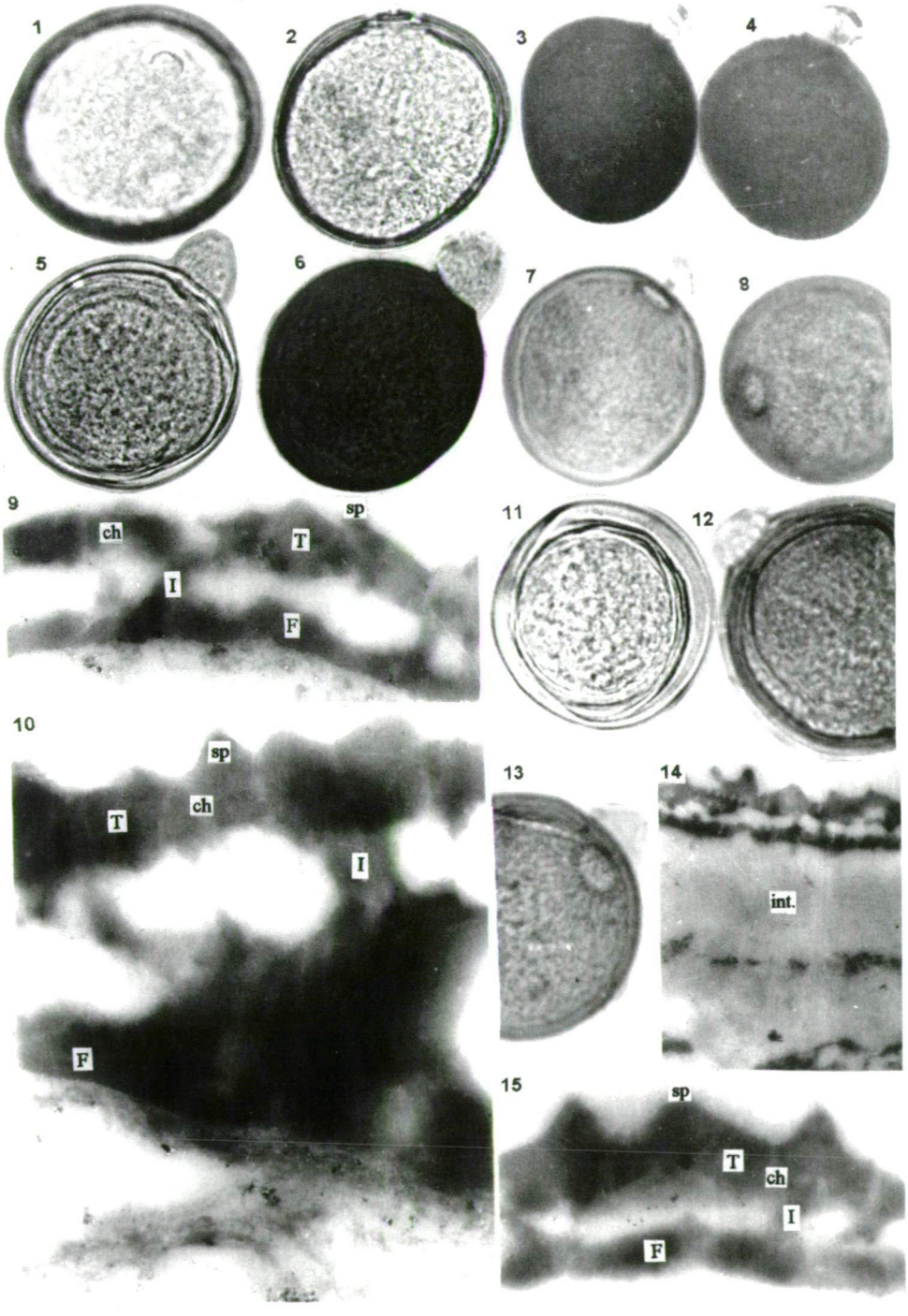

Plate 9.1.

Plate 9.1.

1-15. *Triticum aestivum* L.
1-4. LM pictures. Fresh pollen grains mounted in glycerine-jelly. 1,2. Unstained. 3,4. Stained pollen grains with Methylviolet., 660x.
5-10. Partially degraded pollen grains with 2-aminoethanol (24 hours)
5-8. LM pictures. 5. Unstained. 6. Stained pollen grains with Methylviolet. 7,8. Pollen grains after experimental processes, mounted in Araldite, 660x..
9,10. TEM pictures. 9. Detail from the inter-apertural ectexine. Negative No.: 9017, 33.035x. 10. Exine ultrastructure in the apertural area. Negative No.: 8017, 33.045x.
11-15. Partially degraded pollen grains with 2-aminoethanol (48 hours)
11-13. LM pictures. 11. Unstained. 12. Stained pollen grain with Methylviolet. 13. Pollen grain after experimental processes mounted in Araldite, 660x.
14,15. TEM pictures. 14. Detail from the inter-apertural exine. Negative No.: 9021, 9.910x. 15. Detal from the ectexine ultrastructure. Negative No.: 9023, 33.030x.
T = tectum, I = infratectum, F = foot layer, sp. = spinae, ch. = channel, int. = intine, pr. = protoplasm,

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2.3. Experiment No.: T-12-233, length of time: 72 hours (Plate 9.2., figs. 2-6)
LM results (Plate 9.2., figs. 2-4, table 9.1.) The deformations of the pollen grains started in this experiment. Characteristic wrinkled ectexine appeared in the stained pollen grains. The embedding effect swelled the intine and pro parte the protoplasm. The trend of the alterations of the diameter of the pollen grains is the same as previously.

TEM results (Plate 9.2., figs. 5,6) In the general survey picture of the inter-apertural area, the thickened intine is well illustrated (Plate 9.2., fig. 6). The electron dense ultrastructure of the ectintine is in all probability degraded. Within the more or less homogeneous intine, electron dense granular elements are perceptible, some of them are arranged in a layer. Remnants of the protoplasm are characteristic. In the apertural area (Plate 9.2., fig. 5) the elements of the protrusion are perceptible, but not in the previous preservations. The endannulus and the ectintine are degraded, granular remnants are perceptible. The protoplasmic lamellar elements are also degraded and some electron dense elements are present only.

3. Partial degradation with 2-aminoethanol and $KMnO_4$ aq. dil. (Plate 9.3., figs. 1-12)

3.1. Experiment No.: T-12-234, partial degradation with 2-aminoethanol, and $KMnO_4$ for 24 hours (Plate 9.3., figs. 1-4)

LM results (Plate 9.3., figs. 1,2, table 9.1.) Deformed and wrinkled ectexine was observed in the pollen grains mounted in glycerine-jelly. The "inner body" swelled during the embedding processes. Regarding the diameter of the pollen grains, secondarily larger forms were observed. The maximum is characteristically larger as previous.

TEM results (Plate 9.3., figs. 3,4) The ectexine, in particular the infratectal layer is degraded (Plate 9.3., fig. 4). The ornamental elements of the tectum are characteristic and of different size. In the general survey picture, the degradation of the infratectal layer is well illustrated (Plate 9.3., fig. 3). The remnants of the protoplasm are connected to the foot layer, the elements of the intine are not perceptible.

3.2. Experiment No.: T-12-235, partial degradation with 2-aminoethanol (48 hours) and with $KMnO_4$ (for 24 hours) (Plate 9.3., figs. 5-8)

LM results (Plate 9.3., figs. 5,6, table 9.1) The qualitative alterations are as previously. The trends of the quantitative data of the diameter of the pollen grains are identical with the previous experiment but the values are lesser.

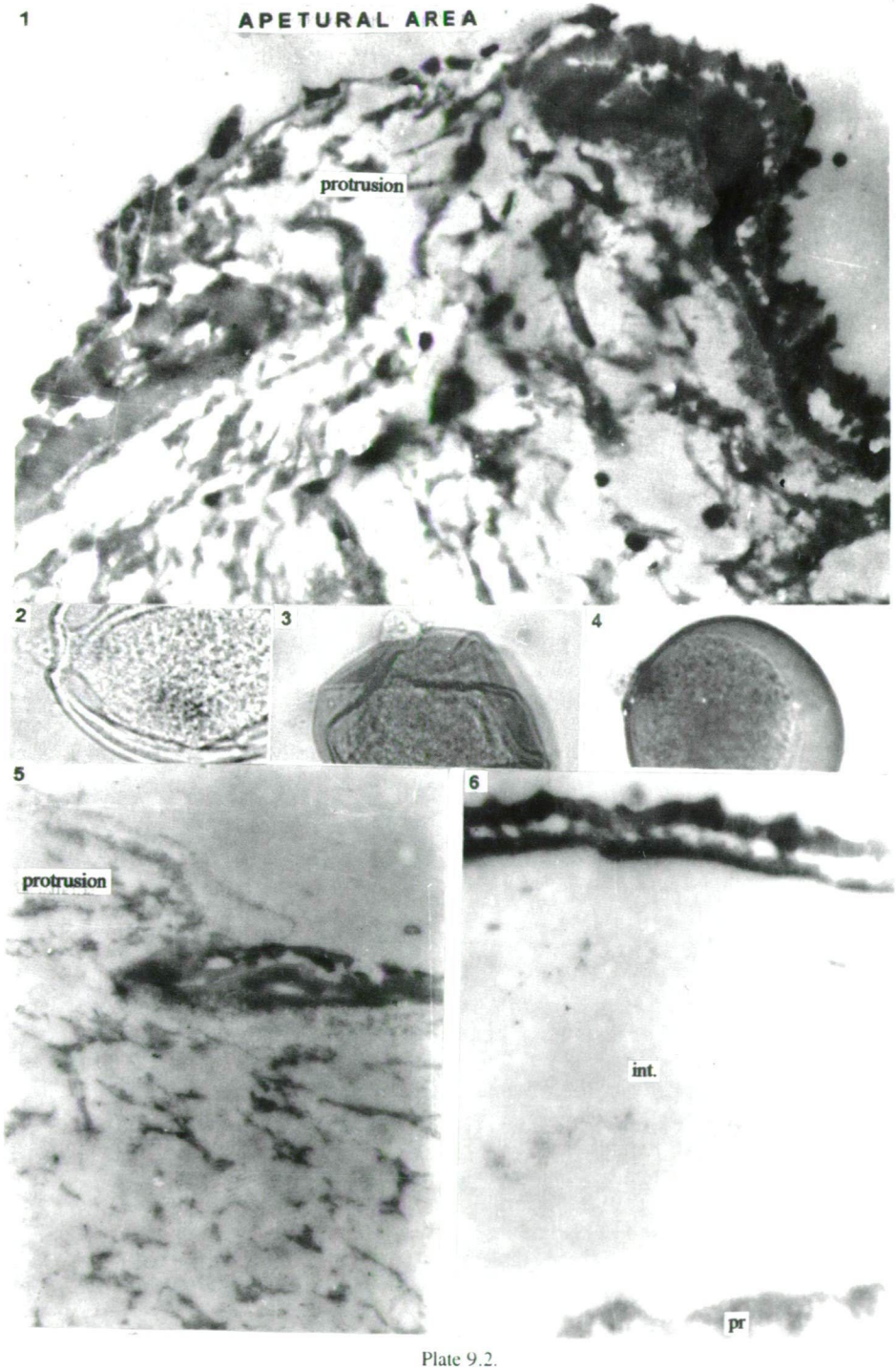

Plate 9.2.

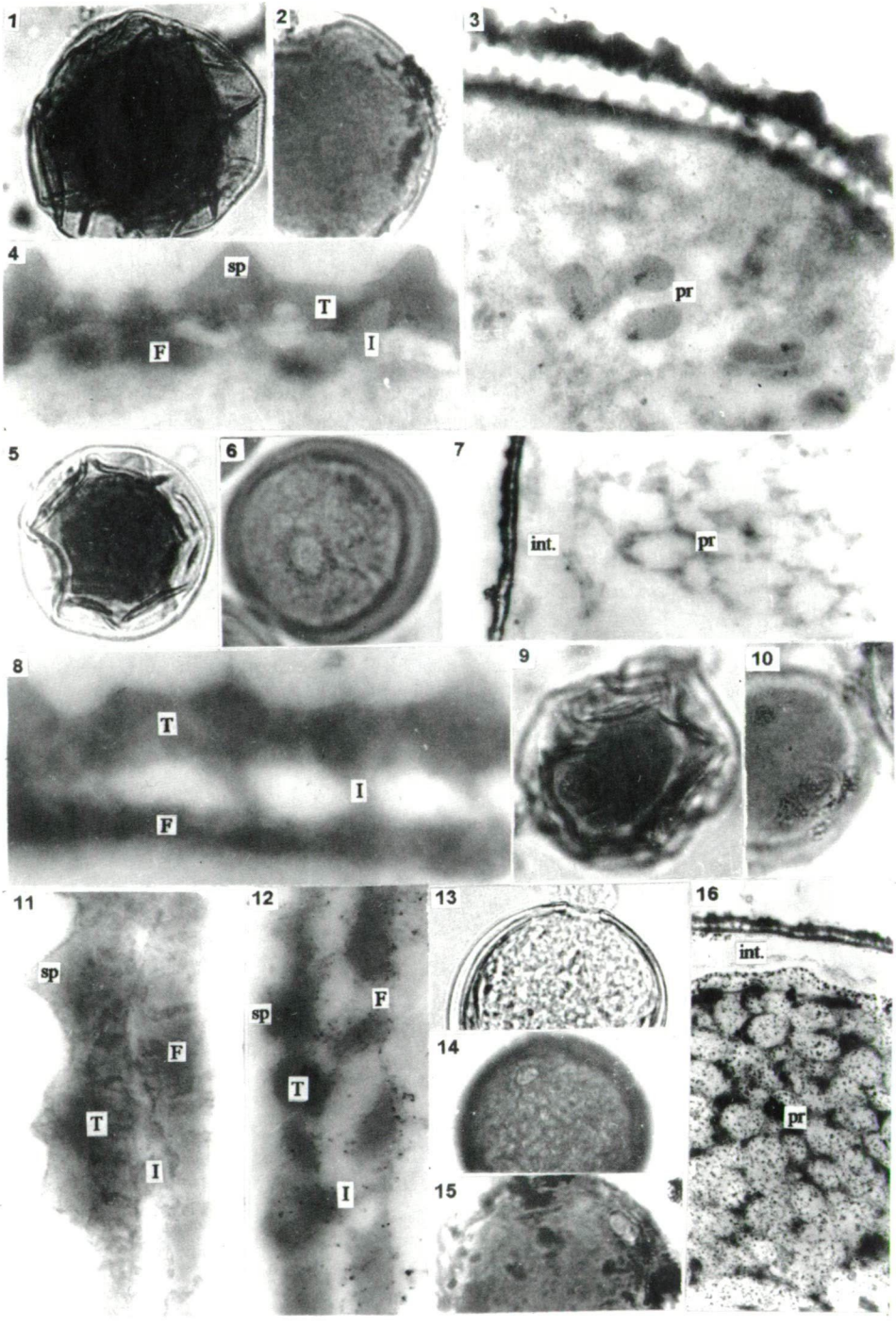
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Plate 9.3.

Plate 9.2.

1-6. *Triticum aestivum* L.
1. Partially degraded pollen grain with 2-aminoethanol (48 hours), ultrastructure of the apertural area. Negative No.: 9028, 9.910x.
2-6. Partially degraded pollen grains with 2-aminoethanol (72 hours)
2-4. LM pictures. 2. Unstained. 3. Stained pollen grain with Methylviolet. 4. Pollen grain after experiment processes mounted in Araldite, 660x.
5,6. TEM pictures. 5. Ultrastructure of the apertural area. Negative No.: 8997, 9.910x 6. Fine structure of the inter-apertural area. Negative No. 9000, 9.910x.

Plate 9.3.

1-16. *Triticum aestivum* L.
1-4. Partially degraded pollen grains with 2-aminoethanol (24 hours) and $KMnO_4$ (24 hours)
1,2. LM pictures. 1. Pollen grain mounted in glycerine-jelly after treatment. 2. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
3,4. TEM pictures. 3. Detail from the ultrastructure of the pollen grain. Negative No.:9008, 9.910x. 4. Detail from the partially degraded ectexine. Negative No.: 9007, 33.035x.
5-8. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with $KMnO_4$ (24 hours)
5,6. LM pictures. 5. Pollen grain mounted in glycerine-jelly after treatment. 6. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
7,8. TEM pictures. 7. General survey picture from the ultrastructure of the pollen grain. Negative No.: 9012, 3289x, 8. Ultrastructure of the partially degraded ectexine Negative No.: 9043, 33.035x.
9-12. Partially degraded pollen grains with 2-aminoethanol (72 hours) and with $KMnO_4$ (24 hours)
9,10. LM pictures 9. Pollen grain after experiment mounted in glycerine-jelly. 10. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
11,12. TEM pictures. Detail from the partially degraded ectexine. 11. Negative No.: 9041, 33.035x, 12. Negative No.: 9044, 33.035x.
13-16. Partially degraded pollen grains with 2-aminoethanol (24 hours) and merkaptoethanol (24 hours)
13-15. LM pictures. 13. Unstained. 14. Stained pollen grain mounted in glycerine-jelly. 15. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
16. TEM picture. General survey picture from the inter-apertural part of the pollen grain. Negative No.: 9064, 3.289x.

TEM data (Plate 9.3., figs. 7,8) In contrast to the previous experiment, remnants of the elements of the intine are relatively well shown. Within the light homogeneous part, a thin electron dense layer is perceptible, probably as a remnant of the ectintine (Plate 9.3., fig. 7). In the degraded protoplasm there are light ellipsoid holes. The ectexine is degraded, channels in the tectum are not perceptible. A trend of homogenization may be observed (Plate 9.3., fig. 8).

3.3. Experiment No.: T-12-236, partial degradation with 2-aminoethanol (72 hours) and with $KMnO_4$ for 24 hours (Plate 9.3, figs., 9-12)

LM results (Plate 9.3., figs. 9,10, table 9.1.) No important changes were observed in the qualitative characteristic features. The maximal value of the diameter of the pollen grains, mounted in glycerine-jelly, is a little smaller than in the previous experiment, but the diameter of the pollen grains mounted in Araldite, is essentially the same.

TEM results (Plate 9.3., figs. 11,12) The degradation of the ectexine is well illustrated it in both pictures. The degradation of the infratectal layer is advanced and sometimes has completely disappeared. Thinning or degradation of the foot layer is also perceptible in some parts of the ectexine (Plate 9.3., fig. 12). Ornamental elements of the tectum are characteristic. Channels were not observed during our investigations. The substance of the ectexine is partially degraded. Electron dense, globular large bio-

polymer units were observed. The arrangement of these units is irregular. It is worth mentioning that biopolymer structures, which are in general present around the channels, were not observed at the sites of the degraded channels.

4. Partial degradation with 2-aminoethanol and merkaptoethanol (Plate 9.3., figs. 13-16, plate 9.4., figs. 1-10)

4.1. Experiment No.: T-12-237, partial degradation with 2-aminoethanol (24 hours) and merkaptoethanol for 24 hours (Plate 9.3., figs. 12-16, plate, 9.4., fig. 1)

LM results (Plate 9.3., figs. 13-15, table, 9.1.) No important qualitative alterations were observed. The stained pollen grains are a little larger than the unstained and the pollen grains mounted in Araldite.

TEM results (Plate 9.3,. fig. 16, plate 9.4, fig. 1) The exine is relatively well preserved. Under the electron dense ectexine, a granular layer is embedded in the light homogeneous part of the intine. Below this layer a darker layer of the intine and another light part is seen. A characteristic layer, composed of electron dense globular units, is beneath this layer, which may be the inner part of the intine. The plasma membrane is electron dense and probably damaged. Organelles of the protoplasm are perceptible, there are vacuoles with tiny electron dense granules.

4.2. Experiment No.: T-12-238, partial degradation with 2-aminoethanol (48 hours) and with merkaptoethanol (24 hours) (Plate 9.4., figs. 2-6)

LM results (Plate 9.4., figs. 2-4, table, 9.1.) The qualitative and the quantitative alterations are essentialy indentical with the previous experiment.

TEM results (Plate 9.4., figs. 5,6) In the inter-apertural area (Plate 9.4., fig. 6) degradation of the ectexine was observed. Thinning of the elements of the infratectal layer and degradation of the superficial ornamental elements are illustrated in picture 6 of the Plate 9.4. Beneath the foot layer, the intine was also degraded. The ultrastructure of the apertural area is relatively well preserved (Plate 9.4., fig. 5). The ectexine seems to be in original preservation except for the inner layers beneath the foot layer. The more or less lamellar or filamentous elements of the protrusion are also perceptible.

4.3. Experiment No.: T-12-239, partial degradation with 2-aminoethanol (72 hours), and with merkaptoethanol for 24 hours (Plate 9.4., figs. 7-10)

LM results (Plate 9.4., figs. 7-9, table 9.1.) Qualitative characteristics are as previously. The maximum of the diameter of the unstained pollen grains is nearly the same in the pollen grains mounted in Araldite as in the previous experiment. Increasing consequences of the stain and embedding effect were observed.

TEM results (Plate 9.4., fig. 10) Degradation of the infratectal layer and the intine is well illustrated. The intine after this experiment is composed of two layers, an outer light and a darker, electron dense part. The plasma membrane is not perceptible. The degradation of the protoplasm is advanced. In the protoplasm there are holes, probably vacuoles.

5. Partial dissolution with glycerine (50%), (Plate 9.4., figs. 11-15)

LM pictures (Plate 9.4, figs., 11-13, table 9.1) Alterations in the quantitative characteristics were observed, in particular the pollen grains mounted in Araldite are smaller than the unstained and stained pollen grains.

TEM pictures (Plate 9.4., figs. 14,15) Degradation of the ectexine was observed (Plate 9.4., fig. 15). More or less radially oriented holes may be the remnants of the channels. Further light globular holes, of irregular arrangement are also perceptible. The infratectal and the foot layers are also damaged. Dark irregular elements are in the degraded intine (Plate 9.4., fig. 14).

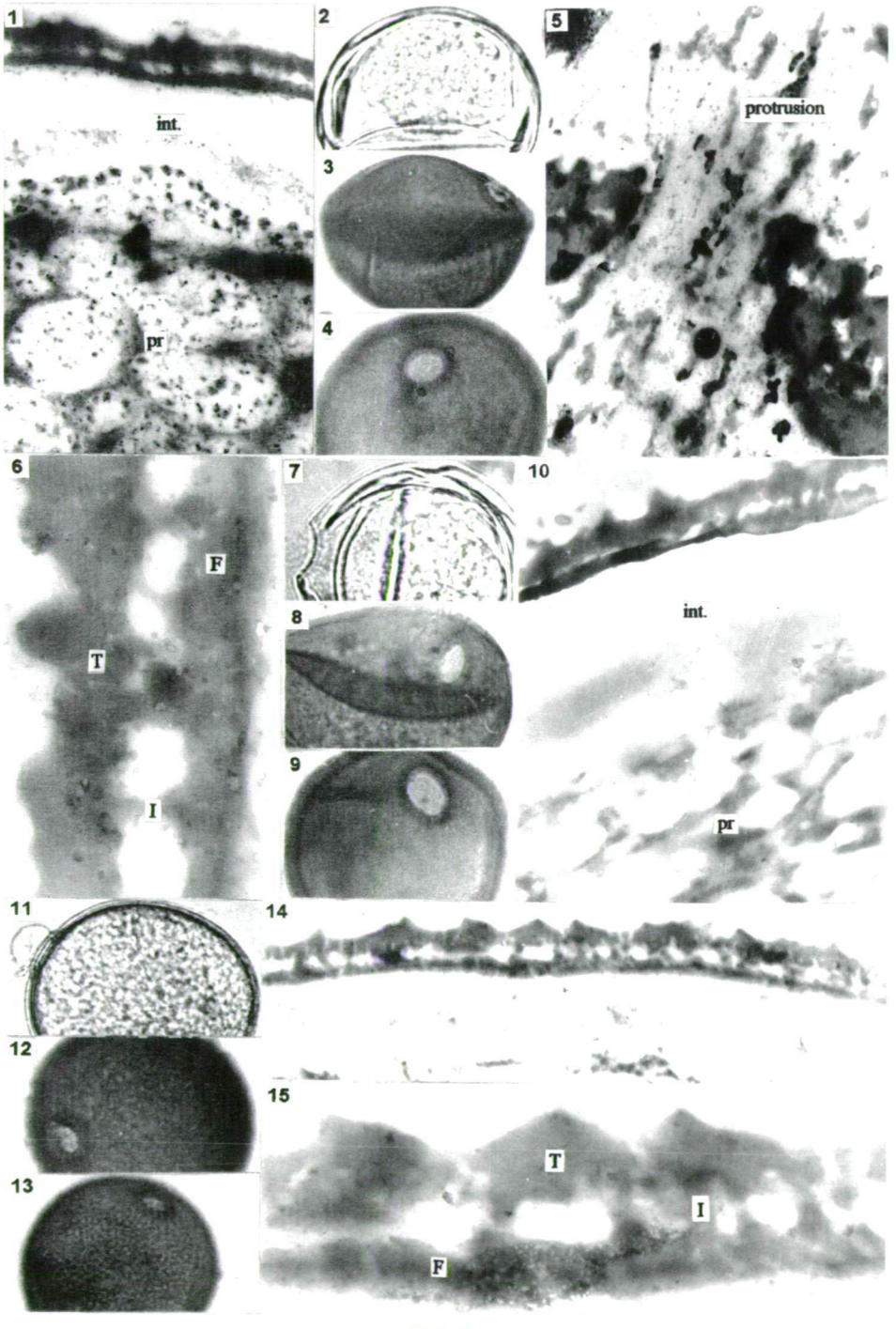

Plate 9.4.

Plate 9.4.

1-15. *Triticum aestivum* L.
1. Partially degraded pollen grain with 2-aminoethanol (24 hours) and merkaptoethanol (24 hours) TEM picture. Detail from the ultrastructure of the inter-apertural part of the pollen grain. Negative No.: 9035, 9.910x.
2-6. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with merkaptoethanol (24 hours)
2-4. LM pictures 2. Unstained. 3. Stained pollen grain with Methylviolet. 4. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
5,6. TEM pictures. 5. Ultrastructure of the apertural area with protrusion. Negative No.: 9049, 9.910x. 6. Detail from the partially degraded ectexine. Negative No.: 9051, 33.035x.
7-10. Partialy degraded pollen grains with 2-aminoethanol (72 hours) and merkaptoethanol (24 hours)
7-9. LM pictures. 7. Unstained. 8. Stained pollen grain with Methylviolet. 9. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
10. TEM picture. Detail of the ultrastructure of the pollen grain in the inter-apertural area. Negative No.: 9196, 9.910x.
11-15. Partially dissolved pollen grains with glycerine (50%) for 30 days
11-13. LM pictures. 11. Unstained. 12. Stained pollen grain with Methylviolet. 13. Pollen grain after experiment and embedding processes, mounted in Araldite, 660x.
14,15. TEM pictures. 14. General survey picture from the interapertural part of the pollen grain. Negative No.: 9053, 9.910x. 15. Detail from the partially degraded ectexine. Negative No.: 9054, 33.035x.

Discussion and Conclusions

Based on our new results we can point out the following:

1. The most important qualitative alterations were observed after the treatment with 2-aminoethanol and $KMnO_4$. The ectexine wrinkled. Orbiculi were not observed during our investigations.

2. As regards the diameter of the pollen grains, the stain altered in a remarkable measure the size of the fresh pollen grains. However in the partially degraded and dissolved pollen grains, a certain regularity was established in the alterations of the diameter of the pollen grains.

3. It is mentioning that the channels of the tectum are not evident after the experiment or have disappeared completely. The sporopollenin molecular system around the channels is less resistant. In contrast, to this, we observed helical biopolymer organization in channels of the tectum of partially degraded pollen grains of *Alnus glutinosa* (KEDVES, SZÉCSÉNYI and SASHALMI, 2002). The channels and the biopolymer organization around them may be important in the diffusion of the allergens from the pollen grains.

4. The sporopollenin of the infratectal layer and the inner lamellar structures in the apertural area are less resistant. In our first observations of fossil angiosperm pollen grains, we observed the degradation of the infratectal layer (KEDVES, STANLEY and ROJIK, 1974).

But our first opinion is that the ontogenetically first developed layer of the ectexine is less resistant was not always supported by our other experiments.

5. The cytoplasmic ultrastructural elements of the protrusions are more resistant than the other part of the protoplasm.

6. The dissolution with diluted glycerine did not reveal the organelles of the cytoplasm as well as we have previously established in the pollen grains of *Platanus hybrida* (KEDVES, PÁRDUTZ and TÓTH, 1999).

Finally, the pollen grains of *Dactylis glomerata* are also included in our research program, and the same experiments will be carried out during the next year. We hope that the comparative evaluation will be interesting and useful.

Acknowledgements

The authors are grateful to Dr. J.F. LAING, Senior Palynologist (Robertson Research International Ltd. Llandudno, U.K.) for critically reviewing the manuscript and for his valuable suggestions. This work was supported by Grant OTKA T 031715.

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10. LM AND TEM INVESTIGATIONS ON EXPERIMENTALLY ALTERED POLLEN GRAINS OF PHOENIX DACTYLIFERA L.

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Abstract

Fresh and partially degraded pollen grains with 2-aminoethanol, $KMnO_4$, merkaptoethanol, and diluted glycerine (50%) were investigated with the LM and TEM method.The new results are the following: 1. The mounted pollen grains were observed in polar position and are opened. 2. Expansion and exudation of the intine and the protoplasm was not observed. 3. The partial degradation revealed the biopolymer system of the sporopollenin not only at the infratectal layer of the ectexine but in the outer and inner part of the foot layer. 4. Regular pentagon biopolymer units were observed. The diameter of these units are characteristically smaller than that of *Phoenix sylvestris* investigated previously. 5. The ultrastructure of the intine particularly in the apertural area is characteristic.

Key words: Experimental Palynology recent, *Phoenix dactylifera.*

Introduction

GRAHAM (1963, p. 36), in his paper concerning the function of pollen wrote the following, "The essential role of pollen grains in plant reproduction has been known for almost 5000 years. The ancient Assyrians were aware that trees of the date palm *(Phoenix dactylifera)* were of two kinds." Later he pointed out, that "Assyrian were not only the first known people to have practiced artificial pollination, but their writings reveal that they considered the pollen-producting plants male and the date-producting plants female." The first LM data of *Phoenix dactylifera* pollen grains were published by WODEHOUSE (1935). Several papers followed this pioneering work, e.g. ERDTMAN (1952), MAHABALÉ (1966), SOWUNMI (1968, 1972) and KEDVES (1980). Multidisciplinary studies were carried out on the palm pollen grains of *Phoenix dactylifera* by BOUGHEDIRI (1988, 1989, 1991, 1999), BOUGHEDIRI and BOUNAGA (1987), BOUGHEDIRI, CERCEAU-LARRIVAL and DORÉ (1995), BOUGHEDIRI et al. (1995), and MANAMANI, BOUGHEDIRI, DOGHMAN and BENOUARTH (2001). Concerning the chemistry of the pollen wall it is worth mentioning the finding published by SHAW and YEADON (1964), p. 247: "ZETZSCHE prepared other pollen membranes in a similar manner and from analytical results suggested that they could be represented by a general molecular formula which is varied from C90H13431 in *Secale cereale* pollen to C90H150O33 for *Phoenix dactylifera*".

During our experimental investigations on Indian palm pollen grains, in particular on the pollen grains of *Phoenix sylvestris* L., we observed a particular organization in the biopolymer structure of the ectexine (KEDVES, BORBOLA, TRIPATHI and MADHAV KUMAR 2000, KEDVES, HORVÁTH, TRIPATHI and MADHAV KUMAR 2001). Further LM data on some partially degraded palm pollen grains from India resulted in interesting alterations in the endexine and the protoplasm (KEDVES, PRISKIN, TRIPATHI and MADHAV KUMAR, 2002).

Taking into consideration the importance of *Phoenix dactylifera* L. and our previous experimental results on recent palm pollen grains, we started a research program on the pollen grains of this species. The first part of our results are presented in this contribution.

Materials and Methods

The pollen grains for our investigations were collected by Dr. Sekina AYYAD (Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt). LM and TEM methods were used. Fresh (T-12-98) and partially degraded pollen grains were investigated as follows:

1. Partial degradation with 2-aminoethanol for 24, 48 and 72 hours (Experiment numbers: T-12-99,100,101). 2. After the degradation with 2-aminoethanol, 10 ml 1% potassium permanganate were added for 24 hours (Experiment numbers: T-12-102, 103, 104). 3. 1 ml merkaptoethanol was added to the partially degraded pollen grains with 2-aminoethanol (Experiment numbers: T-12-105,106, 107). 4. Pollen grains were partially dissolved in glycerine (50%) for 30 days. For every experiment, 5 mg fresh pollen grains were used at temperature 30 °C. For LM studies pollen grains were mounted in glycerine-jelly hydrated at 39.6%, and/or mounted in Araldite after embedding. The ultrathin sections were made with glass knives in the Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, the pictures were taken in the EM Laboratory of the Institute of Biophysics of the Biological Research Center of the Hungarian Academy of Sciences, Szeged. All pictures are unretouched.

Results

1. Fresh pollen grains

LM results (Plate 10.1., fig. 1) In polar position the pollen grains are generally opened, the thinning of the ectexine in the apertural area is well shown. The protoplasm is invaginated in the germinal region. TEM results (Plate 10.1., figs. 2-6) The complete general survey pictures (Plate 10.1., figs. 4, 6) illustrate well the submicroscopic characteristic features of the apertural area of the pollen grain. The colpus and the invaginated protoplasm are well shown. In picture 6 of Plate 10.1., the electron dense ectintine is separate from the foot layer. In highly magnified pictures (Plate 10.1., figs. 2,5) of the perforated tectum, the intine layers are illustrated.

2. Partial degradation with 2-aminoethanol

2.1. Partial degradation for 24 hours (T-12-99) LM results (Plate 10.2., fig. 1) Important alteration as a consequence of this experiment were not observed.TEM results (Plate 10.2., figs. 2-5) Characteristic electron dense granules (microbodies) are in the protoplasm (Plate 10.2., figs. 2,4). Alterations in the ultrastructure of the intine are not uniform. Light intine, sometimes with electron dense granular elements (Plate 10.2., figs. 2,5), or more or less radially oriented electron dense is probably ectintine remnant (Plate 10.2., fig. 4) in the apertural area (Plate 10.2., fig. 2) the electron dense part of the intine and the thin ectexine are well shown. The ectexine is seemingly damaged, the perforations of the tectum are much larger than in the fresh pollen grains (Plate 10.2., fig. 3).

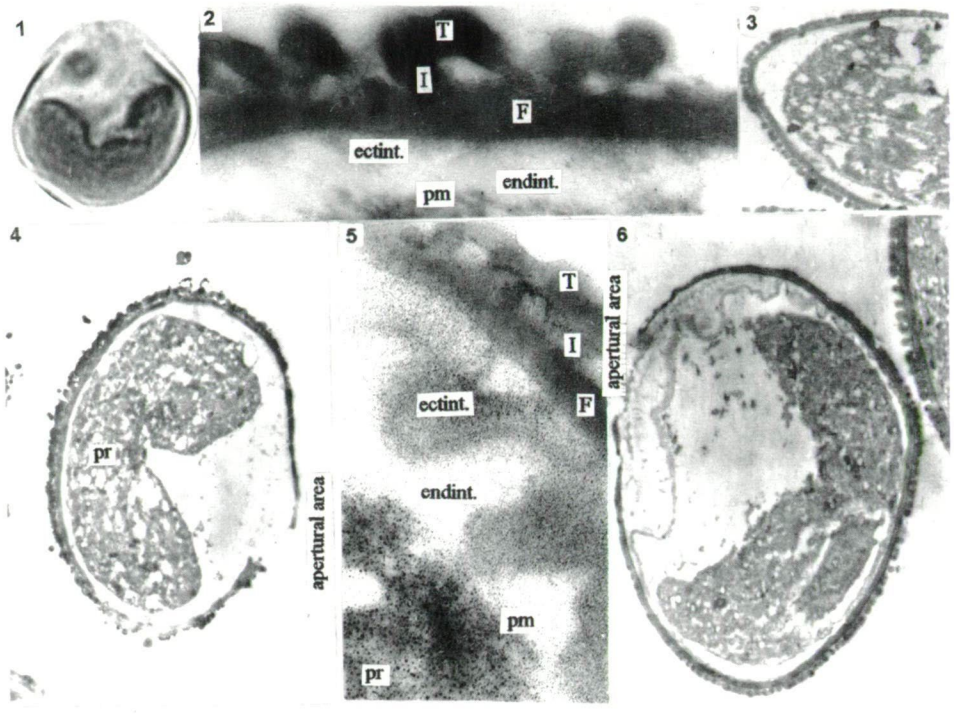

Plate 10.1

1-6. *Phoenix dactylifera* L., fresh pollen grains
1. LM picture, 1650x.
2-6. TEM pictures. 2. Detail from the inter-apertural exine. Negative No.: 8590, 33.035. 3, 4, 6. General survey pictures from the ultrastructure of the pollen grains. 3. Negative No.: 8587, 3.289x, 4. Negative No.: 8754, 3.890x, 6. Negative No.: 8763, 3.890x. 5. Detail from the fine structure of the exine. The ultrastructural characteristic features of the intine are well shown. Negative No.: 8499, 33.035x.
T = tectum, I = infratectum F = foot layer, ectint. = ectintine, endint. = endintine, pm = plasma membrane, pr = protoplasm, mb. = microbody.

Plate 10.2.

1-10. *Phoenix dactylifera* L.
1-5. Partially degraded pollen grains with 2-aminoethanol (24 hours)
1. LM picture. 1650x.
2-5. TEM pictures. 2. General survey picture of the pollen grain. Negative No.:8594, 3.289x, 3. Detail from the fine structure of the ectexine. Negative No.: 8507, 33.035x.. 4. Detail from the ultrastructure of the pollen grains. Negative No.: 8505, 9.910x. 5. Detail from the fine structure of the pollen grains. Negative No.: 8601, 9.910x.
6-10. Partially degraded pollen grains with 2-aminoethanol (48 hours)
6. LM picture 1650x.
7-10. TEM pictures. 7. Detail from the fine structure of the ectexine. Negative No.: 8516, 33.035x. 8,9. General survey pictures from the ultrastructure of the pollen grain. 8. Negative No.: 8602, 3.289x, 9. Negative No.: 8607, 3.289x. 10. Detail from the fine structure of the pollen grain in the apertural area. Negative No.: 8608, 9.910x.

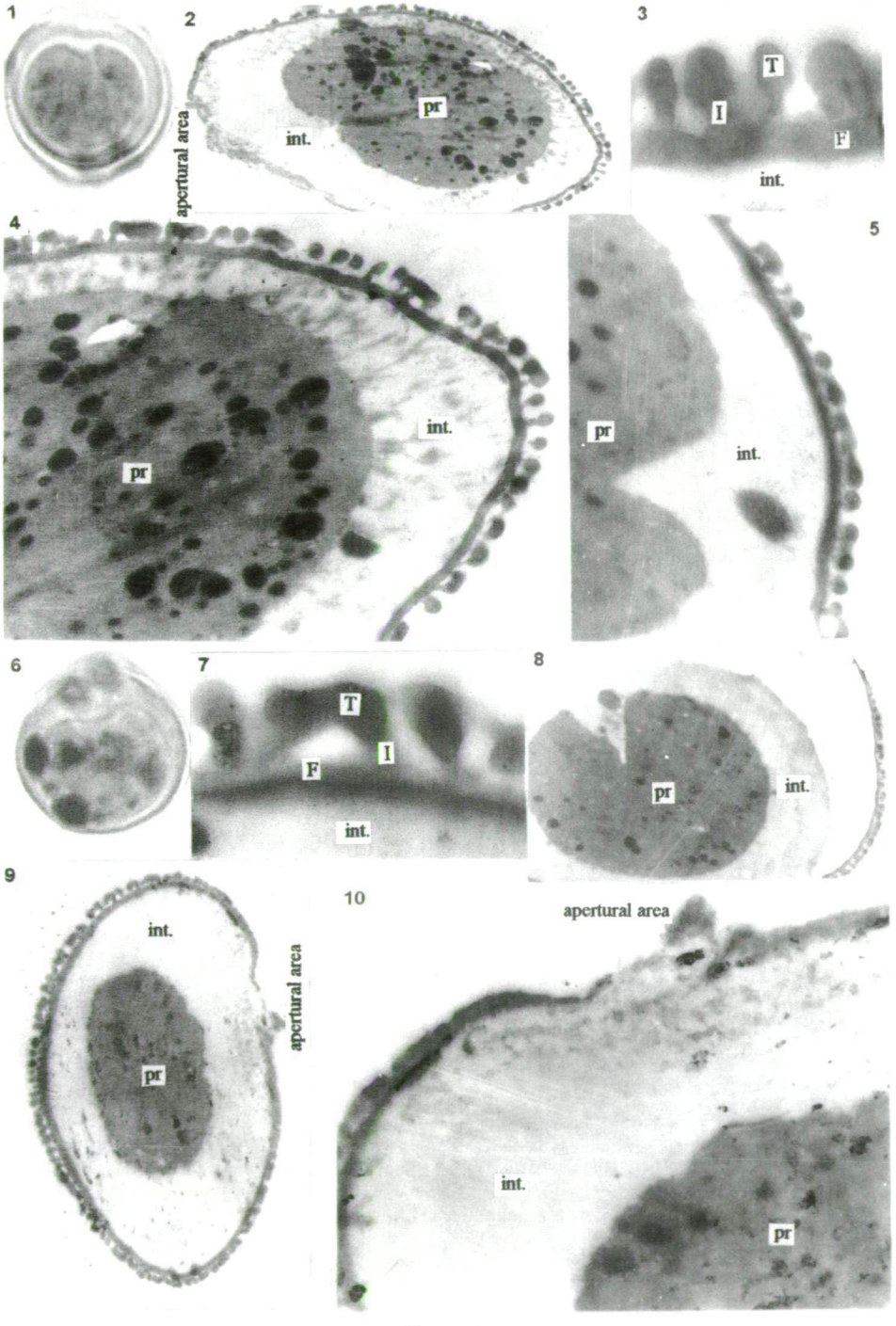
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Plate 10.2.

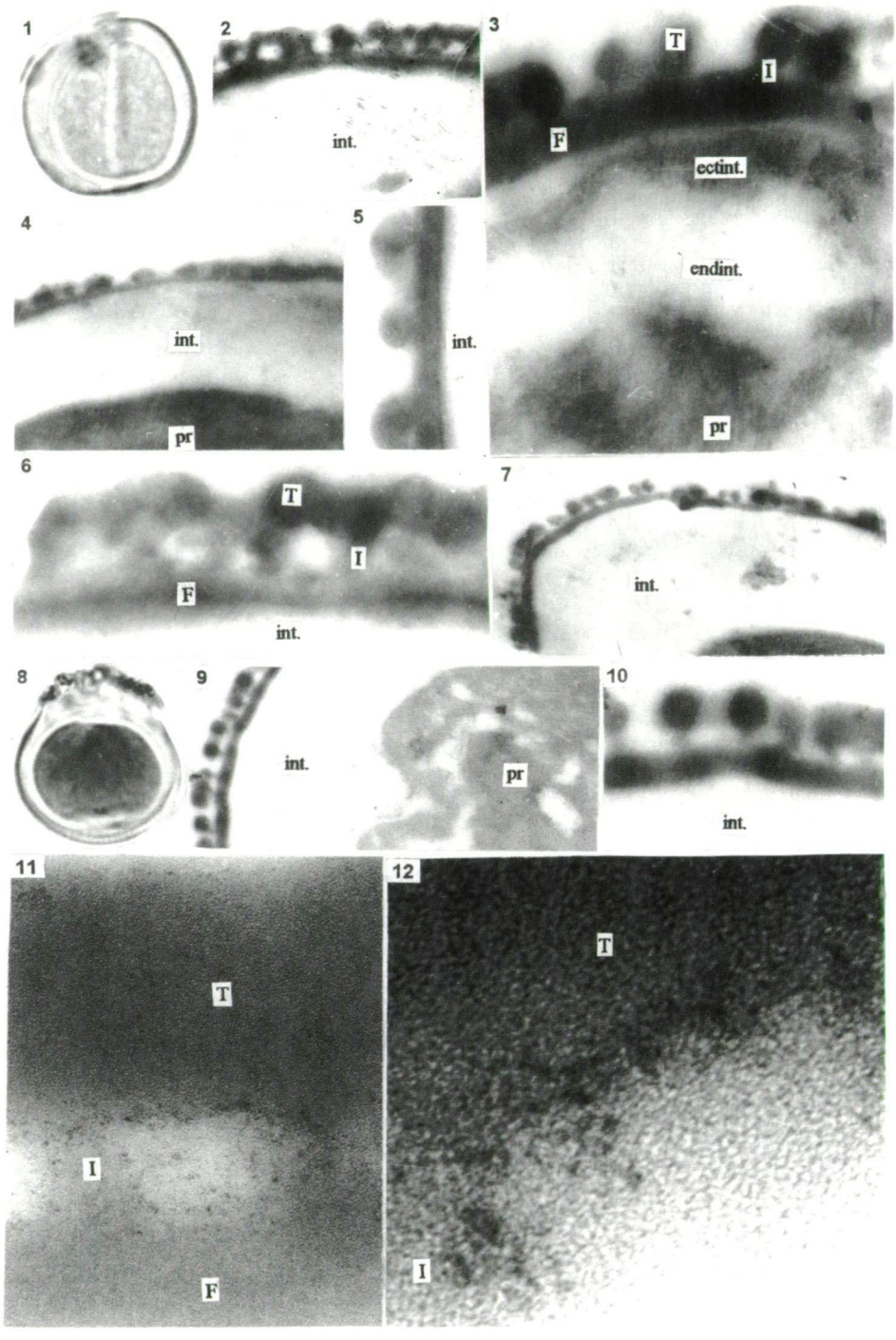
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Plate 10.3.

Plate 10.3.

1-12. *Phoenix dactylifera* L.
1-7. Partially degraded pollen grains with 2-aminoethanol (72 hours)
1. LM picture 1650x.
2-7. TEM pictures. 2-4. General survey picture from the exine ultrastructure of the pollen grain. 2. Negative No.: 8517, 9.910x. 3. Negative No.: 8523, 33.035x. 4. Negative No.: 8528, 9.910x. 5,6. Detail from the ultrastructure of the ectexine. 5. Negative No.: 8529, 33.035x. 6. Negative No.: 8531, 33.035x. 7. Detail from the fine structure of the pollen grain. Negative No.: 8515, 9.910x.
8-12. Partially degraded pollen grain with 2-aminoethanol (24 hours) and with $KMnO_4$ (24 hours)
8. LM picture 1650x.
9-12. TEM pictures. 9. Detail from the ultrastructure of the pollen grain. Negative No: 8578, 9.910x. 10. Ectexine ultrastructure. Negative No.: 8574, 33.035x. 11. Detail from the ectexine ultrastructure. The highly organized biopolymer units of the inner surface of the tectum and the infratectal layer are well shown. Negative No.: 10508, 66.714x. 12. Molecular system of the Inner surface of the tectum and the foot layer. Negative No.:10510, 820.000x.

2.2. Partial degradation for 48 hours (T-12-100). LM results (Plate 10.2., fig. 6) Characteristic electron dense granular elements are in the protoplasm, thinning of the ectexine is perceptible. TEM results (Plate 10.2., figs. 7-10) The ectexine degradation is well shown, the electron dense inner part of the foot layer is significant (Plate 10.2., fig. 7). Disintegration of the intine and the protoplasm are illustrated (Plate 10.2., figs. 8-10), but in the apertural area the outer part of the ultrastructural elements of the intine are resistant e.g.: Plate 10.2., fig. 10.

2.3. Partial degradation for 72 hours (T-12-101). LM results (Plate 10.3., fig. 1) The degradation of the pollen grains is perceptible. TEM results (Plate 10.3., figs. 2-7) The degradation of the ectexine is well shown, in particular in picture 6 of Plate 10.3. Different kinds of alterations in the intine ultrastructure were observed; 1. Electron dense characteristic ectintine and light endintine (Plate 10.3., fig. 3). 2. Not so well characteristic ectintine or more or less homogeneous intine (Plate 10.3., figs. 2,4,7). The plasma membrane seems to be damaged.

3. Partial degradation with 2-aminoethanol and $KMnO_4$

3.1. Partial degradation with 2-aminoethanol for 24 hours and with $KMnO_4$ for 24 hours (T-12-102). LM results (Plate 10.3., fig. 8) The wall of the apertural area and the inner body of the pollen grain is electron dense, the intine is light. TEM results (Plate 10.3., figs. 9-12) The intine is thickened and light, the electron dense elements are probably degraded (Plate 10.3., figs. 9,10). The pictures taken with a high resolution instrument illustrate well the molecular system of the ectexine at different organizational level (Plate 10.3., figs. 11,12). The globular biopolymer units at the infratectal layer and the inner surface of the tectum are well shown.

3.2. Partial degradation with 2-aminoethanol for 48 hours and with $KMnO_4$ for 24 hours (T-12-103). LM results (Plate 10.4., fig. 1) The wall in the apertural area and the inner body of the pollen grain is electron dense, similar to the previous experiment. The size is a little larger than previous. TEM results (Plate 10.4., figs. 2-6) The ultrastructure of the empty pollen grain was also observed (Plate 10.4., fig. 3). In the general survey picture, the light intine and the electron dense granules are well shown in the protoplasm (Plate 10.4., fig. 2). Pictures taken on a high resolution instrument (Plate 10.4., figs. 4-6) illustrate a peculiar process of partial degradation of the ectexine. The discovered biopolymer structures of the infratectal layer and the outer and inner surfaces of the foot layer

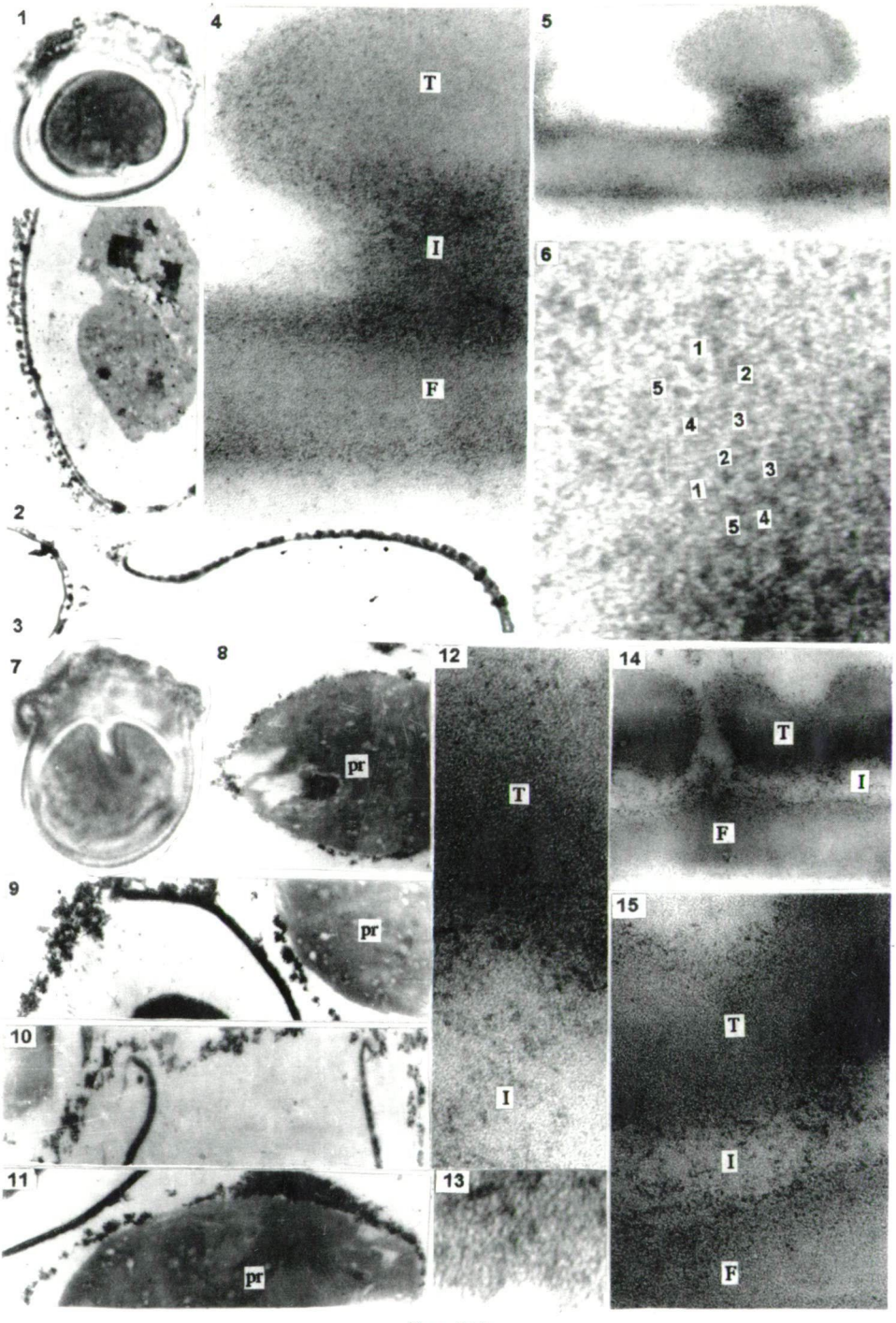

Plate 10.4.

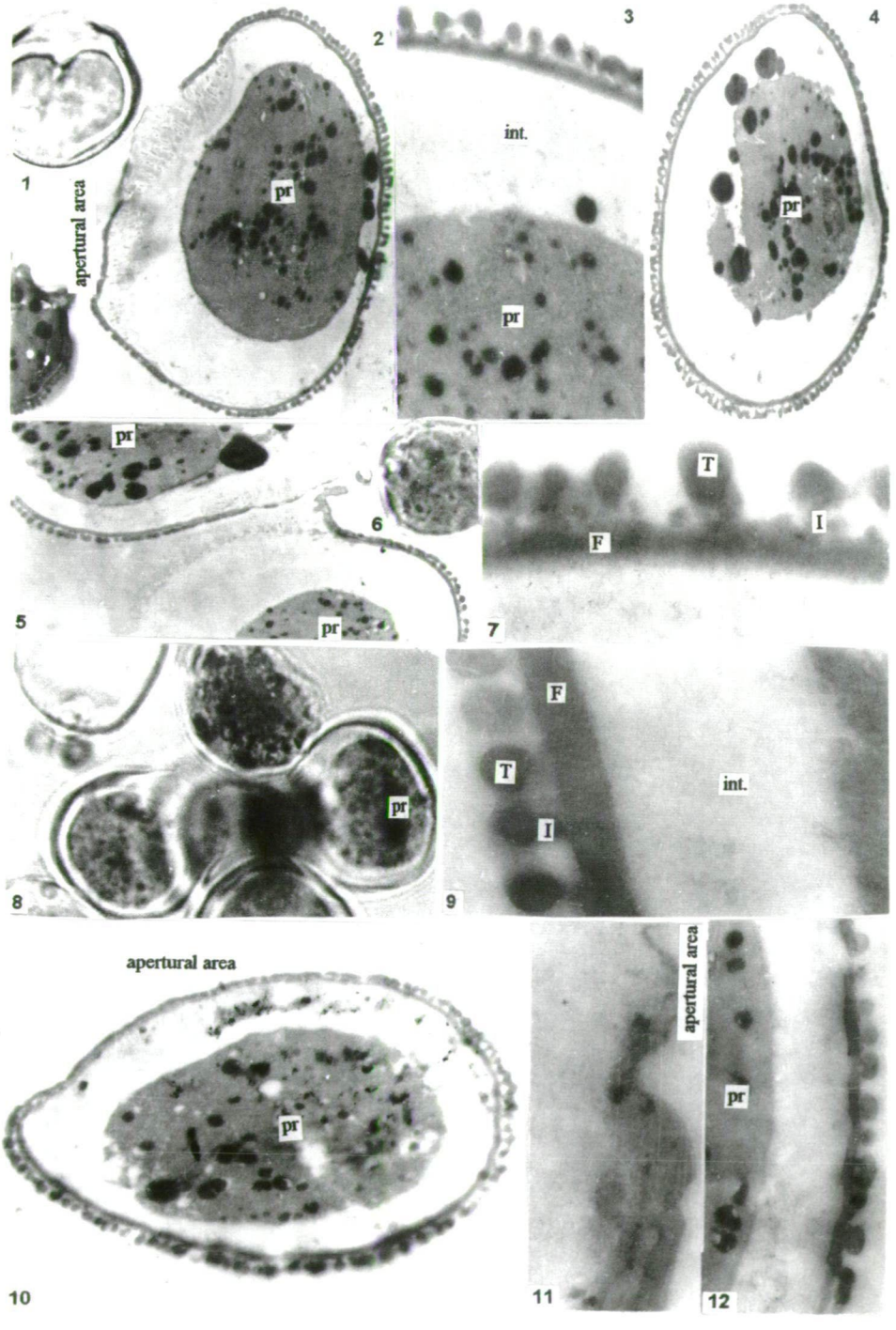
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Plate 10.5.

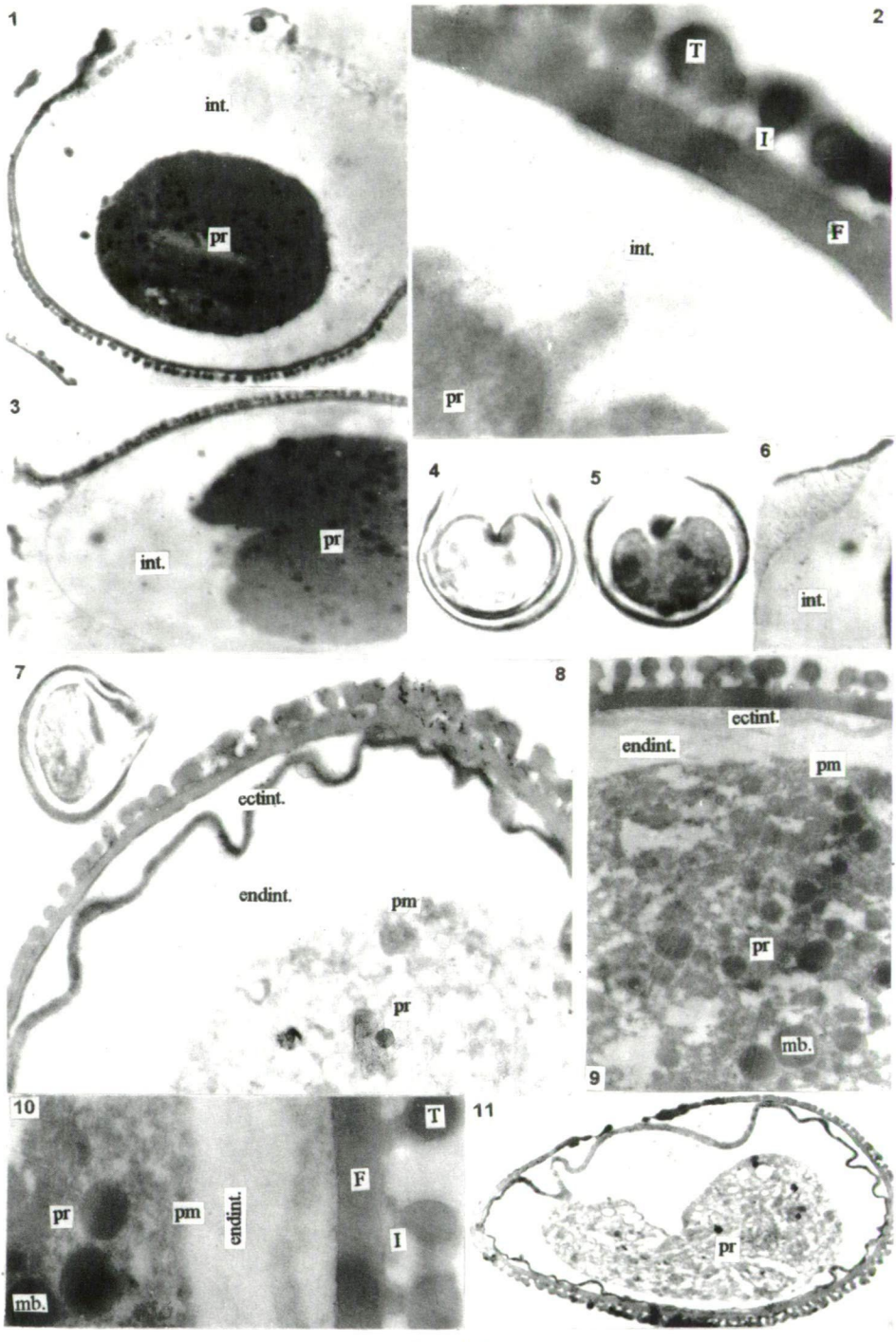
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Plate 10.6.

Plate 10.4.

1-10 *Phoenix dactylifera* L.
1-6. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with $KMnO_4$ (24 hours)
1. LM picture, 1650x.
2-6. TEM pictures. 2. General survey from tthe pollen grains with protoplasm. Negative No.: 8617, 3289x. 3. Detail from the empty pollen grain. Negative No.: 8617, 3289x. 4,5. Detail from the partially degraded ectexine. 4. Negative No.: 10528, 123.000x. 5. Negative No.: 10507, 66.714x. 6. Biopolymer system of the infratectal layer. Negative No.: 10529, 820.000x.
7-15. Partially degraded pollen grains with 2-aminoethanol (72 hours) and with $KMnO_4$ (24 hours)
7. LM picture, 1650x.
8-15. TEM pictures. 8. Detail from the protoplasm of the pollen grain without exine. Negative No.: 8585, 3.289x. 9-11. Detail from the general survey picture of the partially degraded pollen grains. 9. Negative No.: 8581, 3.289x. 10. Negative No. 8583, 3.289x. 11. Negative No.: 8582, 3.289x. 12-15. Detail from the partially degraded ectexine. 12,13. Negative No.:10514, 820.000x. 14. Negative No.: 10513, 33.035x. 15. Negative No.: 10512, 123.100x.

Plate 10.5.

1-12. *Phoenix dactylifera* L.
1-7. Partially degraded pollen grains with 2-aminoethanol (24 hours) and with merkaptoethanol (24 hours)
1,6. LM picture, 1650x.
2-7. TEM pictures. 2,4,5. General survey picture from the partially degraded pollen grain. 2. Negative No.: 8559, 3.289x, 4. Negative No.: 8557, 3.289x. 3. Detail from the ultrastructure of the pollen grain. Negative No.: 8556, 9.910x. 5. Negative No.: 8555, 3.289x. 7. Negaive No.: 8558, 33.035x.
8-12. Partially degraded pollen grains with 2-aminoethanol (48 hours) and with merkaptoethanol (24 hours)
8. LM picture. 1650x.
9-12. TEM pictures. 9-12. Detail from the ultrastructure of the partially degraded pollen grain. 9. Negative No.: 8566, 33.035x, 11. 8569, 33.035x, 12. 8622, 9.910x. 10 General survey picture from the ultrastructure of the pollen grain. Negative No.: 8620, 4780x.

Plate 10.6.

1-11. *Phoenix dactylifera* L.
1-6. Partially degraded pollen grains with 2-aminoethanol (72 hours) and with merkaptoethanol (24 hours)
1-3, 6. TEM pictures. 1,3. General survey pictures from the ultrastructure of the pollen grain. 1. Negative No.: 8568, 3.289x, 3. Negative No.: 8569, 3.289x. 2. Detail from the ultrastructure of the inter-apertural exine. Negative No.: 8629, 33.035x. 6. Detail from the ultrastructure in the apertural area. Negative No.: 8570, 3.289x.
4,5. LM pictures, 1650x.
7-11. Partially dissolved pollen grains with glycerine (50%) for 30 days
7. LM picture. 1650x.
8-11. TEM pictures. 8-10. Details from the ultrastructure of the partially dissolved pollen grain. 8. Negative No.: 8945, 9.910x. 9. Negative No.: 8648, 9.910x. 10. Negative No.: 8649, 33.035x. 11. General survey picture from the ultrastructure of the pollen grain. Negative No.: 8744, 3.289x.

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are well shown. Globular biopolymer units, arranged in regular pentagons, were also observed (Plate 10.4., fig. 6). These pentagons are relatively small, 11-13 Å in diameter.

3.3. Partial degradation with 2-aminoethanol for 72 hours and with $KMnO_4$ for 24 hours (T-12-104). LM results are similar to the previous one (Plate 10.4., fig. 7). TEM results (Plate 10.4., figs. 8-15) The general survey pictures (Plate 10.4., figs. 8-11) illustrate the strong degradation. Pollen grain without ectexine (Plate 10.4., fig. 8), empty pollen grain with degraded ectexine (Plate 10.4., fig. 10) and pollen grains with degraded ectexine (Plate 10.4., figs. 9,11) were observed. In highly magnified pictures

(Plate 10.4., figs. 12-15), the advanced degradation of the tectum is well shown by the globular biopolymer structures.

4. Partial degradation with 2-aminoethanol and with merkaptoethanol

4.1. Partial degradation with 2-aminoethanol for 24 hours and with merkaptoethanol for 24 hours (T-12-105). LM results (Plate 10.5., fig. 1,6) In contrast to the previous series of experiments the inner body is light. In the apertural area, the outer part of the intine is more electron dense than the inner one. TEM results (Plate 10.5., figs. 2-7) In the general survey pictures (Plate 10.5., figs. 2-5) the following may be pointed out: 1. There are dark globular units (microbodies) in the protoplasm and sometimes in the inner part of the intine (Plate 10.5., figs. 2-4). 2. The electron dense part of the outer part of the intine is also well shown in these pictures (Plate 10.5., fig. 2). Degradation of the ectexine was also observed (Plate 10.5., fig. 7).

4.2. Partial degradation with 2-aminoethanol for 48 hours and with merkaptoethanol for 24 hours. (T-12-106). LM results (Plate 10.5., fig. 8): The dark granules in the protoplasm are well shown in this picture. TEM results (Plate 10.5., figs. 9-12). These results are essentially identical with the previous experiments.

4.3. Partial degradation with 2-aminoethanol for 72 hours and with merkaptoethanol for 24 hours (T-12-107). LM results (Plate 10.6., figs. 4,5): The dark granular units in the protoplasm are well shown after staining (Plate 10.6., fig. 5). TEM results (Plate 10.6., figs. 1-3, 6) The outer part of the intine in the apertural area is less electron dense (Plate 10.6., fig. 1). In several places, there is an outer thin layer ot the intine in the apertural region (Plate 10.6., fig. 6). The microbodies are present in the intine and in the protoplasm.

5. Partial dissolution with diluted glycerine (50%) for 30 days (T-12-108)

LM results (Plate 10.6., fig. 7) The pollen grains after this experiment were not completely opened as with the previous ones. TEM results (Plate 10.6., figs. 8-11) The general survey pictures illustrate interesting alterations in the electron density and the swelling of the ectintine (Plate 10.6, figs. 8,11). The protoplasm, including the plasma membrane, is well preserved (Plate 10.6., figs. 9,10). Plasma membrane, microbodies and sometimes mitochondria were observed.

Discussion and Conclusions

1. Concerning the LM data, we can point out that, the peculiar swelling and exudation of the protoplasm and intine, which was observed previously (KEDVES, PRISKIN, TRIPATHI and MADHAV KUMAR, 2002) on Indian palm pollen grains, was not observed in the present investigations.

2. The discovery of the biopolymer structure of the ectexine is different from the pollen grains of *Phoenix sylvestris*, but the biopolymer system of both *Phoenix* species investigated up till now, is particular. The regular pentagons in *Phoenix sylvestris* are unusually large, those of *Phoenix dactylifera* are small.

3. After dissolution with diluted glycerine, the protoplasmic organelles are relatively well preserved, similar to our first observations on the pollen grains of *Platanus hybrida* BROT. (KEDVES, PÁRDUTZ and TÓTH, 1999).

Finally, further experimental investigations will be carried out, we hope that the partial degradation using the C60 fullerene/benzol solution will bring interesting new data concerning biopolymer organization of the ectexine.

Acknowledgements

We are grateful to Dr. Sekina AYYAD (Department of Botany, Faculty of Science, Mansoura University, Mansoura, Egypt), and to Dr. J.F. LAING Senior Palynologist (Robertson Research International Ltd. Llandudno, U.K.) for critically reviewing the manuscript and for his valuable suggestions. This work was supported by Grant OTKA T 031715 and PRCH Student Science Foundation. DT 2001. május 5., DT 2001, nov./ 2.

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11. SYMMETRY OPERATIONS ON THE C60 FULLERENE/BENZOL SOLUTION REVEALED BIOPOLYMER STRUCTURES

M. KEDVES, O. BÉRES, D. JACSÓ, I. KOCSICSKA and B. VARGA

Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, H-6701, P.O. Box 993, Szeged, Hungary

Short communication

The first TEM data were published previously on partially degraded wall of *Botryococcus braunii* KÜTZ. with C60 fullerene/benzol solution (KEDVES and FREY, 2002). Later this solvent was applied for different kind of recent pollen grains. During our experimental investigations on the pollen grains of *Taxus baccata* L. (KEDVES, PÁRDUTZ, JACSÓ, KOCSICSKA and VARGA, under publication) one experiment revealed different kinds of biopolymer units in angstrom dimension. Regular hexagon connected with a regular pentagon was also observed. Our first results on the symmetry operations in this subject are summarized as follows:

1. The verification of the symmetry of the hexagonal biopolymer unit, connected with a pentagon was succesful (Plate 11.1.). Several secondary points of symmetry appeared, which may be used for further symmetry operations.

2. Five and tenfold primary rotation was used for another single, regular pentagon (Plate 11.2.). Secondary rotations were also used (cf. KEDVES, 1989). The alterations of the rotation areas are more or less regular. Worth of mentioning that the secondary rotations have not resulted the Penrose unit. In this way it may be presumed that the regular pentagon without connections may be a component of a disintegrated large biopolymer structure of C60 fullerene type.

Finally, we may emphasize, that the first attempts concerning the symmetry operations of the biopolymer structures, revealed with the C60 fullerene/benzol solution was succesful.

This work was supported by Grant OTKA T 031715.

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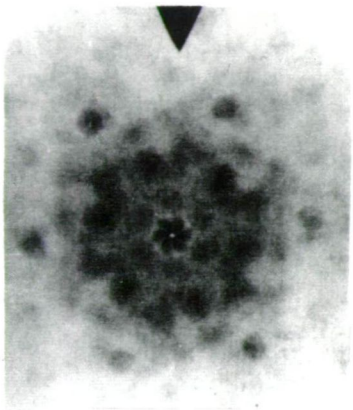
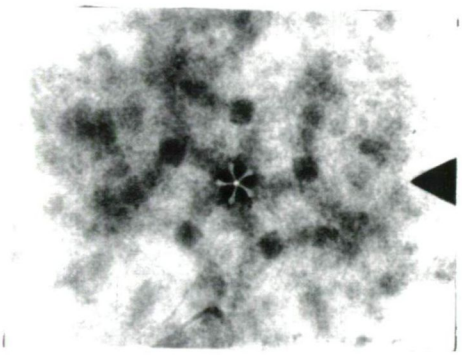
KEDVES, M. and FREY, K. (2002): C60 fullerene/benzol solution as an agent of partial degradation of *Botryococcus braunii* KÜTZ. colonies from Hungarian Alginite. – Plant Cell Biology and Development (Szeged) *14*, 92-94.

KEDVES, M., PÁRDUTZ, Á., JACSÓ, D., KOCSICSKA, I. and VARGA, B. (2002): C60 fullerene/benzol solution in the partial degradation of the pollen grain wall of *Taxus baccata* L. – under publication.



C.P.6.A.6.6.



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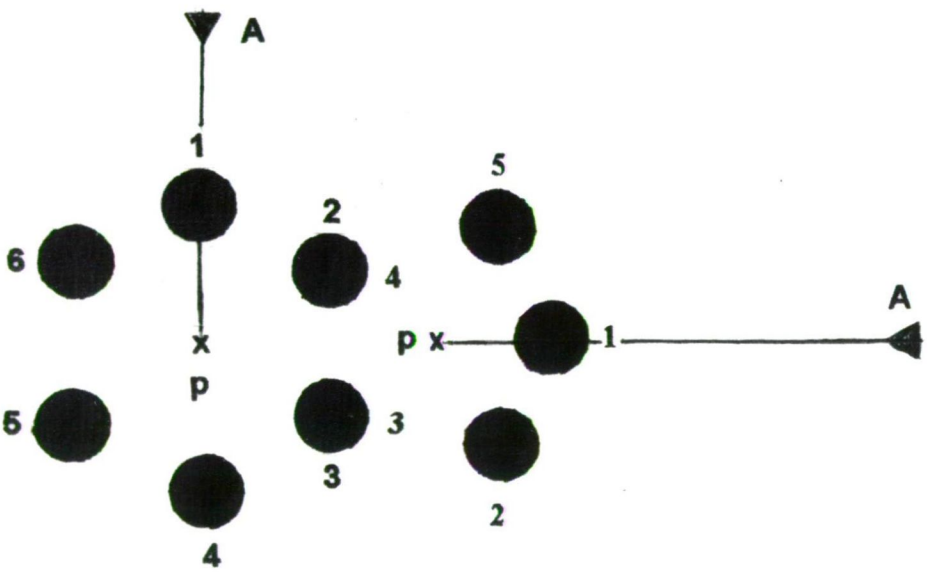

Plate 11.1

Scheme of the hexagonal biopolymer unit connected with a regular pentagon and the axes of rotation. We emphasize the importance of the two common biopolymer units of the hexagon and pentagon. (Magnification of this schema: 2.5 Million).
The six- and tenfold primary rotation pictures. N : 500.000x. Worth of mentioning are the secondary points of symmetry.

Plate 11.2.

Scheme of another single regular pentagon of 2.5 Million magnification. Fivefold and tenfold primary rotations, and altogether six secondary rotation pictures of 500.000 magnification are presented herein. There are several secondary points of symmetry are illustrated.

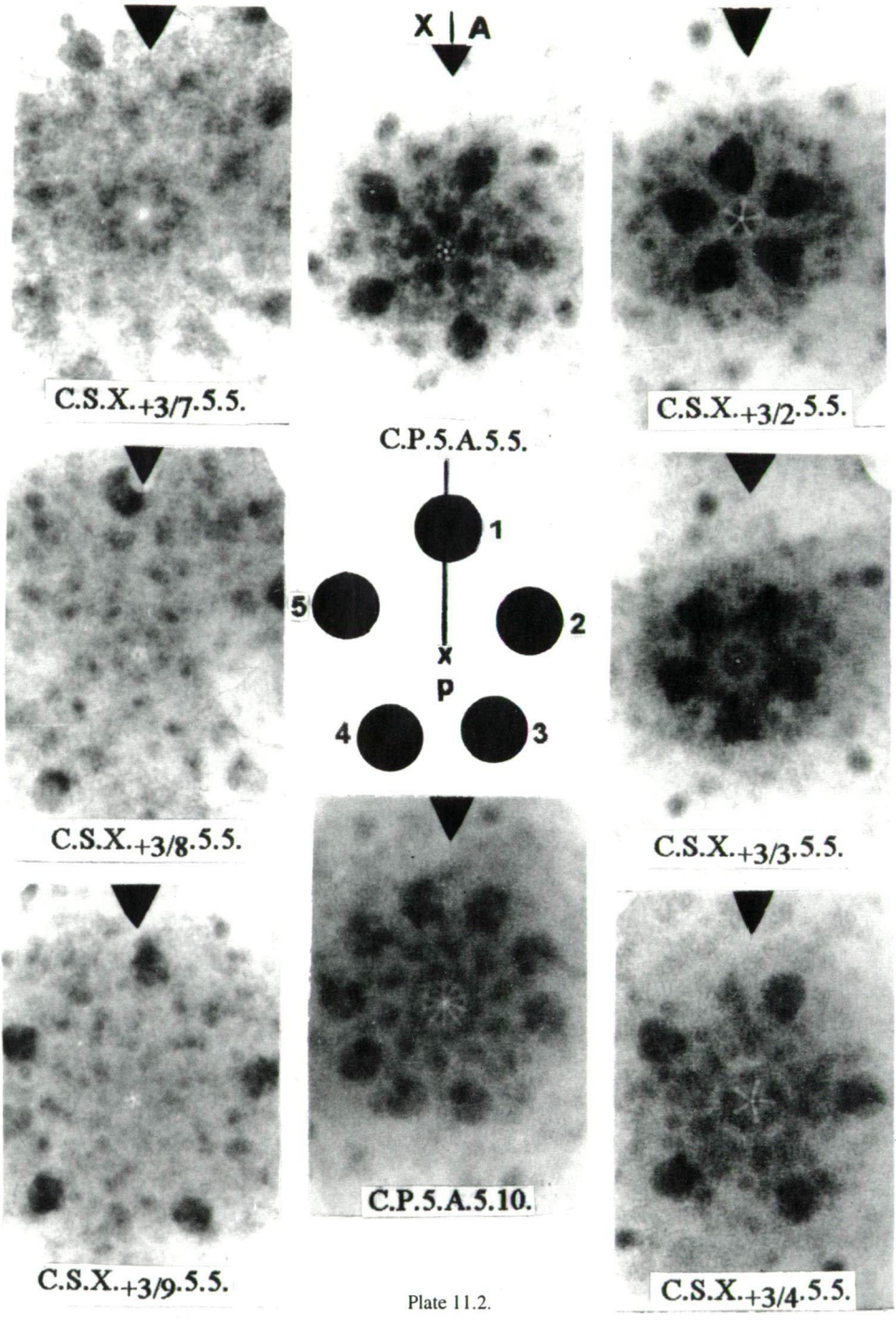
X | A
C.S.X.+3/7.5.5.
C.P.5.A.5.5.
C.S.X.+3/2.5.5.
1
2
3
4
5
X
P
C.S.X.+3/8.5.5.
C.S.X.+3/3.5.5.
C.P.5.A.5.10.
C.S.X.+3/9.5.5.
C.S.X.+3/4.5.5.

Plate 11.2.

12. SYMMETRY OPERATIONS ON OCTAGONAL BIOPOLYMER STRUCTURE OF PARTIALLY DEGRADED ECTEXINE OF GINKGO BILOBA L.

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Short communication

During our TEM investigations on partially degraded ectexine of *Ginkgo biloba* L. different kinds of biopolymer structures were observed. For the first time a probably octagonal unit was also observed. By the eightfold rotation we verified the symmetry of this unit. Moreover, in highly magnified pictures the molecular system of the globular units were also observed. This organization is new and we hope for interesting results.

Now we have summarized a very short review selected from the previous publications of our Laboratory concerning the biopolymer structures. The first globular biopolymer structures in fossil angiosperm ectexines revealed during the sedimentation processes (KEDVES, STANLEY and ROJIK, 1974). Further first application on the protoplast method (*Helix* enzyme) to the pollen grains of *Corylus avellana* (KEDVES, 1976) was used. Then after the first five-fold rotation of a regular pentagon biopolymer was observed (KEDVES, 1988). Basic for the methods for two dimensional symmetry operations was explained by KEDVES (1989a). Thereafter, first synthesis of the quasi-crystalloid biopolymer structures and its highly organized degrees by KEDVES (1989b). The three dimensional modelling of the quasi-crystalloid biopolymer system was also illustrated for the first time by KEDVES (1991). The first computer modelling for the quasi-crystalloid biopolymer structure (KEDVES and KEDVES, 1995) and biopolymer structures revealed with C60 fullerene/benzol solution (KEDVES and FREY, 2002).

Finally, we need to emphasize here that, the biopolymer system of the sporoderm seems to be more complicated as we believed earlier. The composition and the molecular organization depends on several factors and it is difficult to solve its organization with an unique model.

This work was supported by Grant OTKA T 031725.

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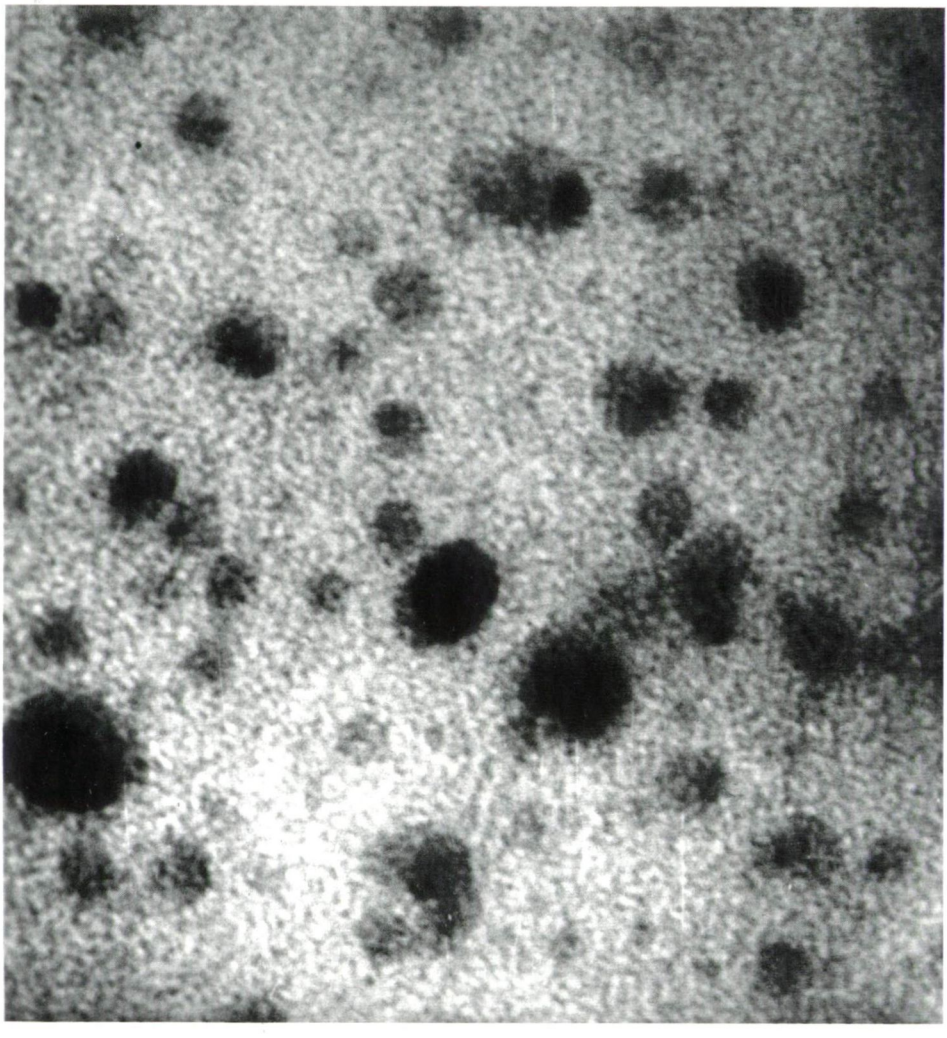

Plate 12.1.

Ginkgo biloba L. Biopolymer structure of the partially degraded ectexine with 2-aminoethanol for 24 hours and with $KMnO_4$ aq. dil. 1% for 24 hours. Experiment No.: T-12-220. Negative No.: 11730, 1,000.000x.

Plate 12.2.

Ginkgo biloba L. C.P.8.A.8.8. rotation picture of the octogonal biopolymer unit. C.P.8.A.8.8. The cyclic molecular structure of the electron dense biopolymer units are also well shown 1,600.000x.

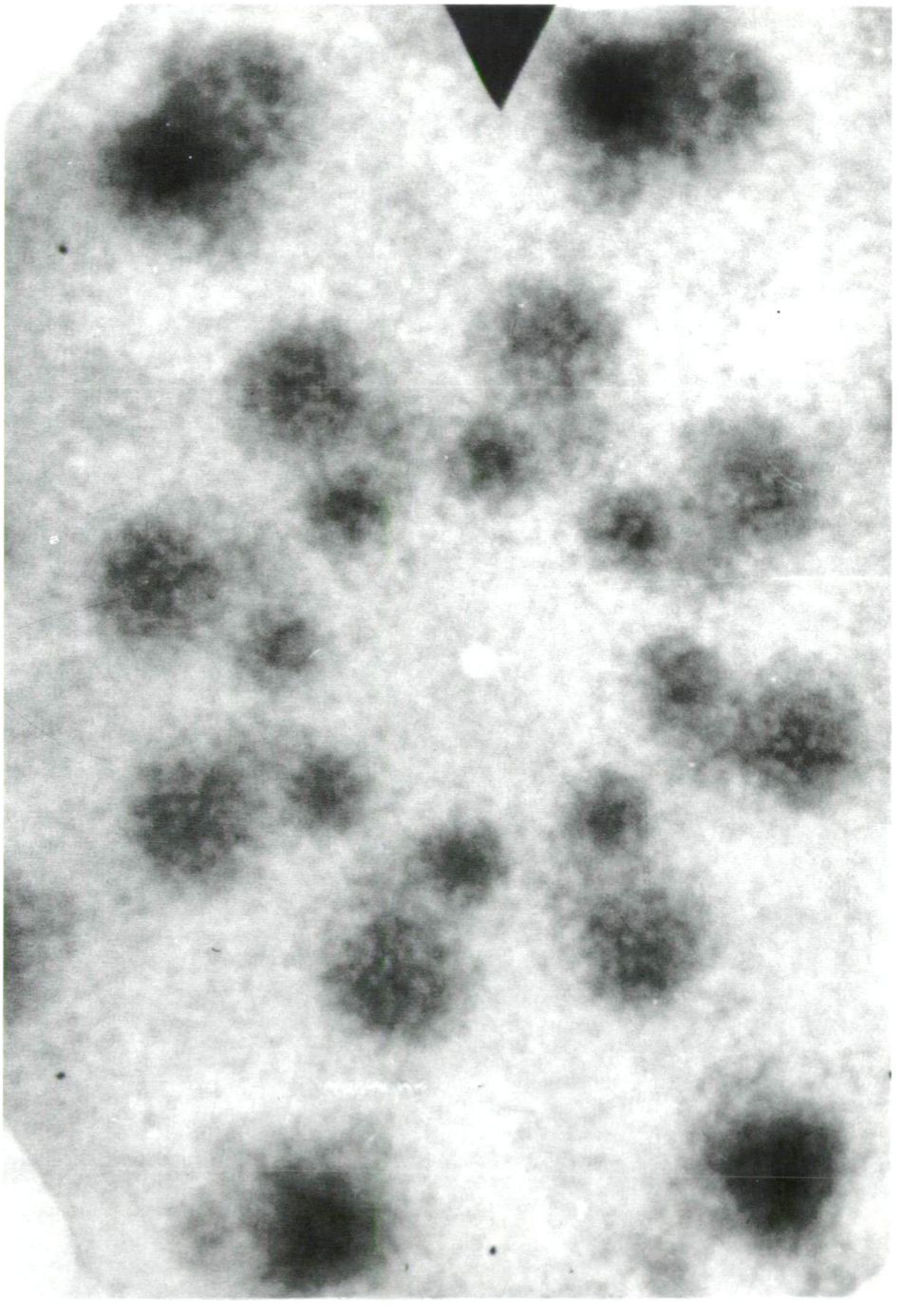

Plate 12.2.

13. LIST OF PUBLICATIONS OF THE LABORATORY UNTIL DECEMBER 2002

Compiled by

O. BÉRES and L. JACSÓ

Cell Biological and Evolutionary Micropaleontological Laboratory of the University of Szeged, H-6701, P.O. Box 993, Szeged, Hungary

BAJPAI USHA (2002): Comparison of the ultrastructure of the cuticle in some extimct and extant taxa of gymnosperms from India. - Plant Cell Biology and Development (Szeged) *14*, 17-24.

KEDVES, M. (2002b): Ultrastructure of plant microfossils in Mesozoic amber. - 8th Int. Symposium on Mesozoic Terrestrial Ecosystems, Cape Town, South Africa, Abstracts, 30.

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KEDVES, M., SASHALMI, J. and SZÉCSÉNYI, A. (2002): Symmetry operations on the quasi-periodic biopolymer structures of the wall of *Botryococcus braunii* KÜTZ. - Plant Cell Biology and Development (Szeged) *14*, 85-91.

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Chronicle

compiled by

D. JACSÓ and L. JACSÓ

Visiting scientist

Dr. MADHAV KUMAR Scientist 'D', Birbal Sahni Institute of Palaeobotany, Lucknow worked in our Laboratory under the Exchange of Scientist Program between the Indian National Science Academy and the Hungarian Academy of Sciences. During his visit between 8th September - 16th October, 2002 (Plate 1, figs 2, 5), we worked on the SEM results of partially degraded Malvaceae pollen grains and on the combined (TEM and SEM) investigations on partially degraded cuticles of *Cycas rumphii*. Further research works were started such as, the combined studies on the partially degraded pollen grains of *Cycas rumphii* MIQ. using the C60 fullerene/benzol solution.

International Laboratory activities and news

28 January - 10 February, 2002, Lucknow, Uttar Pradesh, India

Prof. Dr. M. KEDVES visited Birbal Sahni Institute of Palaeobotany, Lucknow. During his visit on 30th January, Prof. Dr. A.K. SINHA, Director of the Birbal Sahni Institute of Palaeobotany was awarded with the Millenium Medal of the Laboratory (Photo 1,2). The program of the function was following: 16.00 hours -Welcome and introduction of Prof. KEDVES by the Director. 16.10 hours - About Hungarian Academy by Prof. KEDVES. 16.20 hours - Medal Award Ceremony. Introduction and presentation of medal by Prof. Kedves to Director BSIP. 16.30 hours -Thanks by the Director, BSIP. 16.40 hours - BSIP Association with Hungary by Dr. S.K.M. TRIPATHI. 16.50 hours -Vote of thanks by Seniormost Scientist, Dr. SHAILA CHANDRA. After this ceremony a reception was held in the garden of the BSIP. The moments of this occasion was published in daily Newspapers - Rashtriya Sahara (local Hindi daily), Indian Express (English daily) and also in Newsletter of Birbal Sahni Institute of Palaeobotany for information to public and academic institutions.

Dr. M. KEDVES, Dr. S.K.M. TRIPATHI and Dr. MADHAV KUMAR finalized two manuscripts on the pollen grains and the preliminary report on the cuticles of *Cycas rumphii* MIQ., and worked on the further research programs on the partially degraded pollen grains of two species of the Malvaceae pollen grains. The new programs were also included. Fruitful discussions were carried out with Dr. USHA BAJPAI, on her new contributions in P.C.B.D.

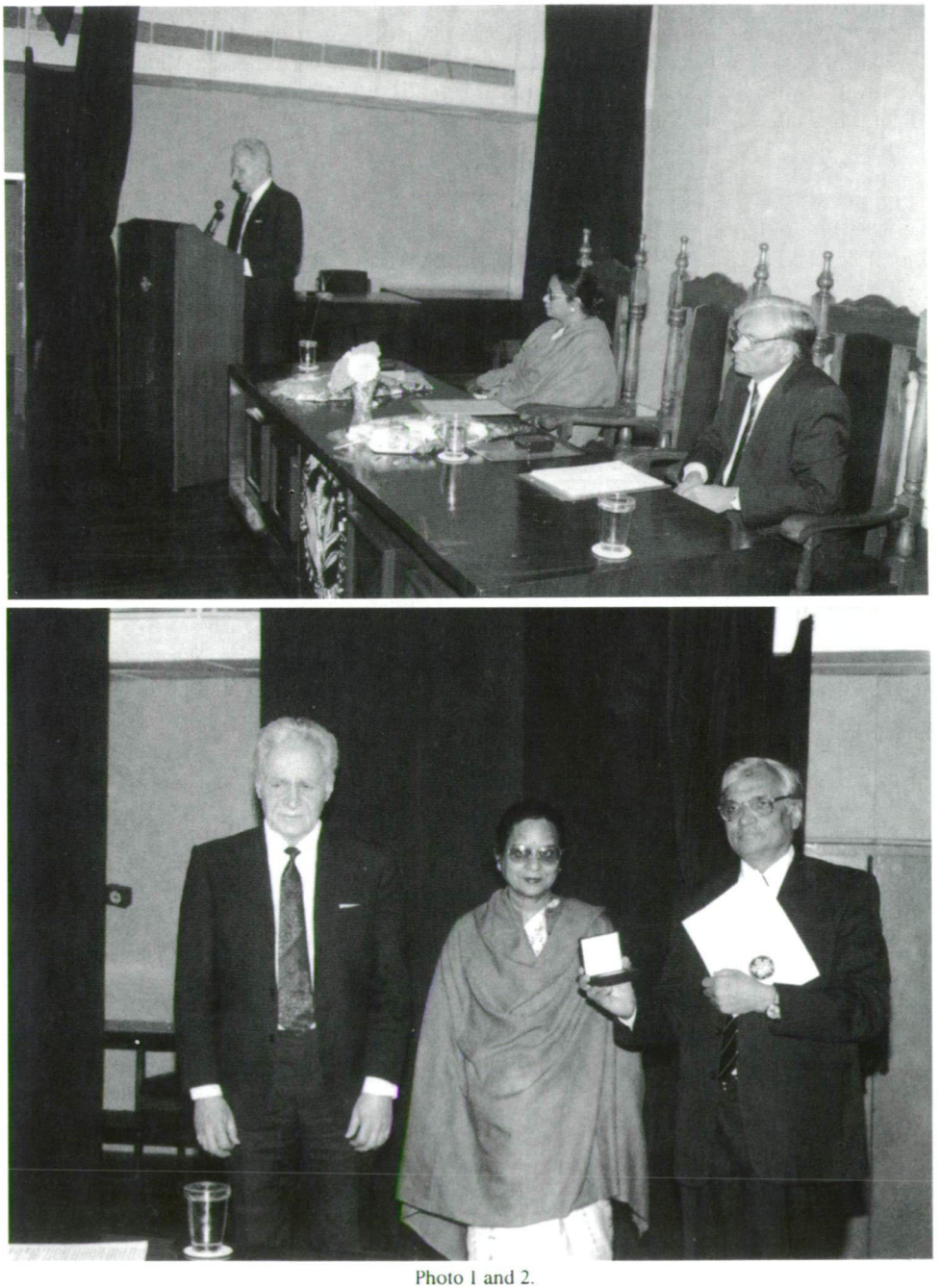

Photo 1 and 2.

From right to left: Prof. Dr. A.K. SINHA Director, Dr. SHAILA CHANDRA, Seniormost Scientist and Prof. Dr. M. KEDVES in the Auditorium of the Birbal Sahni Institute of Palaeobotany, Lucknow, India.

On the 8th February an exclusive reception was held in the room of Dr. SHYAM C. SRIVASTAVA at the appearance of the first number of Savitriana. Editors: H. A. KHAN, R. SAXENA, S.C. SRIVASTAVA. Advisory Board: Prof. G.V. PATLL, Ex-Vice-Chancellor, Amravati University, Amravati, Maharashtra, Mr. RAVI SHANKAR, Ex-Director General, G.S.I., Kolkata, Prof. SAEED A. SIDDIQI, Ex-Chairman, Botany Department, A.M.U. Aligarh, U.P., Prof. R.Y. SINGH, Geology Department, Punjab University, Chandigarh, Punjab, Prof. MOHAMMAD IQBAL, Chairman, Botany Department, Jamia, Hamdard, New Delhi, Prof. C.G.K. RAMANUJAM, Ex-Chairman, Botany Department, Osmania University, Hyderabad, Dr. A.N. PATHAK, Director, Council of Science and Technology, Lucknow, U.P., Prof. M. KEDVES, C.B.E.M. Lab. Botany Department, J.A. University, Szeged, Hungary, Dr. LI-CHEN SEN, Institute of Botany, Beijing, China, Dr. Edith TAYLOR, Botany Department, University of Kansas, St. Louis, Kansas, USA, Dr. Ruth STOCKEY, Botany Department, University of Alberta, Edmonton, Canada, Dr. Joanna VAN CITTERT, Department of Palaeobotany and Palynology, Konigcenburg, The Netherlands, Dr. TRIN TZANH, Director, Geological Museum, Hanoi, Vietnam.

21 - 26 July, 2002, Cape Town, South Africa, 8th International Symposium on Mesozoic Terrestrial Ecosystems. Organizing Committee: Dr. Roger SMITH, Prof. Anusuya CHINSAMY-TURAN, Dr. Sanghamitra RAY, Co-ordinators: Sally ELLIOTT, Anne GREENHILL.

On the 25 July M. KEDVES delivered the following lecture:

Ultrastructure of plant microfossils in Mesozoic amber.

29 August - 2 September, 2002. Athens, Greece, 6th European Paleobotany-Palynology Conference. Organizing Committee: President: E. VELITZELOS, Vice president: M.D. DERMITZAKIS, General Secretary: E. GEORGIADES-DIKEOULIA, Treasurer: K. KYRIAKOPOULOS, Members: A. ANTONARAKOU, C. DOUKAS, H. DRINIA, CH. IOAKIM, V. KARAKITSIOS, E. KOSKERIDOU, K. KOULI, S. PAVLIDES, F. POMONI, M. TRIANTAPHYLLOU, N. TSAPARAS, TH. TSOUROU. Special advisors: TH. DENK, D. VELITZELOS.

On 30th August M. KEDVES delivered the following lecture:

Trends in the investigations of the biopolymer structure of the sporoderm.

Based on the letter of Prof. Dr. TSENG-CHIENG HUANG dated 2001, 07, 23, from 1st August 2001, Prof. Dr. SU-HWA CHEN appointed Editor-in-Chief of Taiwania. At this occasion a picture taken in the Botanical Garden of Nanjing at the 10th International Palynological Congress is presented in this volume (Photo 3).

A picture taken during the ICPC, in the Conference Building is enclosed herein (Photo 4), Dr. HUANG FEI, (Nanjing Institute of Geology and Paleontology Academia Sinica, Nanjing, P.R. of China) and Dr. M. KEDVES.

Photo 3.

From right to left: Prof. Dr. SU-HWA CHEN, Prof. Dr. M. KEDVES, Dr. MEI-HUEI TSENG (National Taiwan Science Education Centre), Miss HUEI-SING HUANG (Graduate student of Dept. of Geology, NTU).

Photo 4.

DR. HUANG FEI and Prof. DR. M. KEDVES, at the 10th I.P.C.

Photo 3.

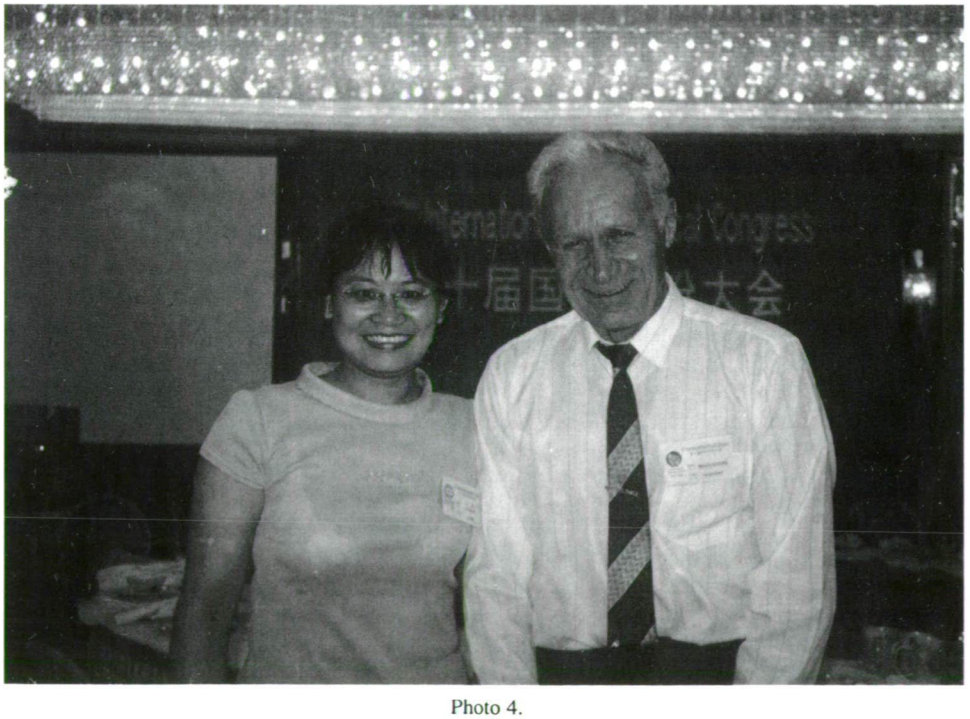

Photo 4.

Hungarian scientific activities

During the 1380th meeting of the Botanical Section of the Hungarian Biological Society on the 15th April, 2002 the following paper was presented by M. KEDVES:

KEDVES, M., TOMBÁCZ, D. - SZÉCSÉNYI, A.: Kisérletes vizsgálatok a búza virágporszemein.

On the 2nd December (1336th meeting) another paper was presented by M. KEDVES - Növényi biopolimer rendszerek kutatási irányzatai - at the Botanical Section of the Hungarian Biological Society.

Laboratory meetings and news

26.01.02. The manuscripts for the volume 15 of the Plant Cell Biology and Development were arranged in order for publication . The research, scientific and other academic activities in the Laboratory were organized with its actual problems.

16.02.02. Report of the scientific achievements of the joint research programs with the Birbal Sahni Institute of Palaeobotany, Lucknow (India) was prepared and discussed with the colleagues. A discussion on the present state of the international scientific research programs was also made.

06.04.02. Discussions on the participation in international scientific meetings (Cape Town and Athens).

27.04.02. The compilation of the manuscripts for the next volume (15) of the Plant Cell Biology and Development. Methodical problems in the investigations of the biopolymer system of the different kinds of plant cell walls were made in record. The importance of the C60 fullerene/benzol solution in the discovery of different organization level of the biopolymer structures was discussed. Further, it is decided to continue the experiments with this solution.

01.06.02. Discussion with the Rector of the University concerning the present day problems of the Laboratory was also conveyed to the students. Further experiments were done on different kinds of pollen grains with C60 fullerene/benzol solution and observed the results in transmission electron microscope.

19.08.02. At 4 Pm an exclusive reception was held in the Laboratory (Plate 1, figs. 1,3,4). Participants: A. BORBOLA, D. JACSÓ, L. JACSÓ, M. KEDVES, K. PRISKIN and D. TOMBÁCZ.

07.09.02. Report of the participation in the international scientific meetings at Cape Town and Athens was prepared along with the present day programs of the Laboratory.

05.10.02. The final state of the 15th volume of Plant Cell Biology and Development was made and discussed the scientific programs for 2003 with students.

02.11.02. The research works of the Laboratory within the present joint research programs with the Birbal Sahni Institute of Palaeobotany, Lucknow, India was organized.

07.12.02. Review of the achievements of the Laboratory during 2002 was prepared along with discussions on the problems and programs for 2003.

Plate 1.

1. Participants of the Laboratory Meeting in the office of Prof. Dr. M. KEDVES. From left to right: A. BORBOLA, PhD. Student, Prof. Dr. M. KEDVES, D. JACSÓ, L. JACSÓ, K. PRISKIN and D. TOMBÁCZ.
2. DR. MADHAV KUMAR, Scientist 'D' working on the manuscript of one of the joint research program.
3. L. JACSÓ and D. JACSÓ in the Laboratory at the exclusive reception.
4. A. BORBOLA in the Laboratory after the award ceremony.
5. Dr. MADHAV KUMAR, Scientist 'D' in the office of PROF. DR. M. KEDVES.

The pictures are taken by Dr. É. SIPOS-KEDVES.

Plate 1.

Teaching program of the Laboratory

In the year 2002, the following lectures were delivered:

1. Applied Palynology 2 + 2, Biopolymer organization and symmetry 2 + 0, 3. Theory of Evolution and Natural Phylosophy, 2 + 0, 4. Introduction to the Pre-Quaternary Palynology, 2 + 2, 5. Theory of the Supernova, 2 + 0, 6. Basic Palynology 2 + 2, 7. Quasi-crystalloid biopolymer structures 2 + 0.

Award

Prof. Dr. M. KEDVES was awarded "The American Medal of Honor" by the American Biographical Institute (Photo 5). The number of his medal is 57. Worldwide 100 persons were selected for this honor.

Photo 5

Front picture of the American Medal of Honor. The picture is taken by Dr. I. BAGI.

Responsible for publications: M. KEDVES
Responsible editors: D. JACSÓ and L. JACSÓ
Cover by K. PRISKIN
Set in New Times 10/11

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